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L. EDELEN
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13281 U.S.PTO

Mail Stop Provisional Patent Application

PTO/SB/16 (6-95)
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Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE

PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53 (c).

	Docket Number	620-285	Type a plus sign (+) inside this box→	+

INVENTOR(S)/APPLICANT(S)

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031356 U.S.PTO
60/531979
122403

TITLE OF THE INVENTION (280 characters)

EP_2 AGONISTS

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒	Specification	Number of Pages	58	☐	Applicant claims "small entity" status. ☐ "Small entity" statement attached.
☒	Drawing(s)	Number of Sheets	4	☐	Other (specify)

METHOD OF PAYMENT *(check one)*

☒	A check or money order is enclosed to cover the Provisional filing fees ($160.00)/($80.00)	PROVISIONAL FILING FEE AMOUNT ($) 160.00
☒	The Commissioner is hereby authorized to charge any deficiency, or credit any overpayment, in the fee(s) filed, or asserted to be filed, or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Account No. 14-1140. A duplicate copy of this sheet is attached.	

The invention was made by an agency of the United States Government or under a contact with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,
SIGNATURE B. Sadoff DATE December 24, 2003

TYPED or PRINTED NAME B. J. Sadoff

REGISTRATION NO. *(if appropriate)* 36,663

☐ Additional inventors are being named on separately numbered sheets attached hereto.

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804102

EP_2 RECEPTOR AGONISTS

This invention relates to certain stereoisomers of AH13205, (±)- *trans*-2-[4-(1-hydroxyhexyl)phenyl]-5-oxo-cyclopentaneheptanoic acid and their use as EP_2 receptor agonists. The invention also relates to pharmaceutical compositions comprising these stereoisomers, and the use of these stereoisomers and compositions to treat various diseases.

Background to the invention

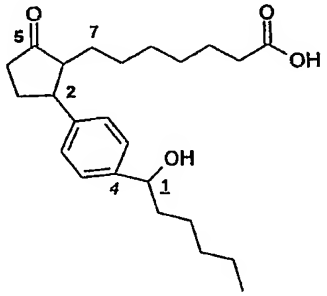
Prostanoids comprise prostaglandins (PGs) and thromboxanes (Txs) and their receptors fall into five different classes (DP, EP, FP, IP and TP) based on their sensitivity to the five naturally occurring prostanoids, PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and TxA_2, respectively (Coleman, R.A., Prostanoid Receptors. *IUPHAR compendium of receptor characterisation and classification*, 2nd edition, 338-353, ISBN 0-9533510-3-3, 2000). EP receptors (for which the endogenous ligand is PGE_2) have been subdivided into four types termed EP_1, EP_2, EP_3 and EP_4. These four types of EP receptors have been cloned and are distinct at both a molecular and pharmacological level (Coleman, R.A., 2000)

EP_2 agonists have been shown to be effective in the treatment of a number of conditions, including (but not limited to) dysmenorrhoea (WO 03/037433), pre-term labour (GB 2 293 101), glaucoma (WO 03/040126), ocular hypertension (WO 03/040126), immune disorders (WO 03/037433), osteoporosis (WO 98/27976, WO 01/46140), asthma (WO 03/037433), allergy (WO 03/037433), bone disease (WO 02/24647), fracture repair (WO 98/27976, WO 02/24647), male sexual dysfunction (WO 00/40248), female sexual dysfunction (US 6,562,868), periodontal disease (WO 00/31084), gastric

ulcer (US 5,576,347) and renal disease (WO 98/34916).

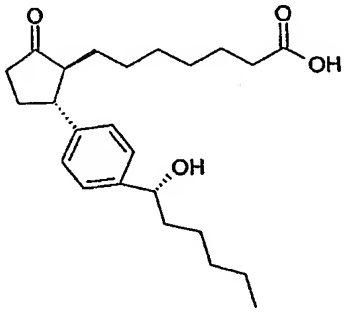
AH13205, (±)- *trans*-2-[4-(1-hydroxyhexyl)phenyl]-5-oxo-cyclopentaneheptanoic acid, is known as an EP_2 agonist (for example, see Hillock, C.J. and Crankshaw, D.J., *European Journal of Pharmacology*, **378**, 99-108 (1999)).

It can also be called (7-{**2**-[*4*-(1-hydroxy-hexyl)-phenyl]-**5**-oxo-cyclopentyl}-heptanoic acid (fonts added for identification), and has the following structure:

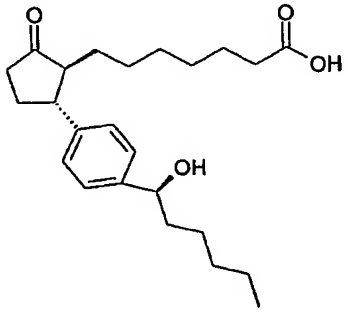


This structure has three chiral carbon atoms and hence eight possible stereoisomers. When the groups on the cyclic pentanone are in a *trans* relationship, this gives rise to four stereoisomers which are the major ones and when the groups are in a *cis* relationship, gives rise to four minor stereoisomers.

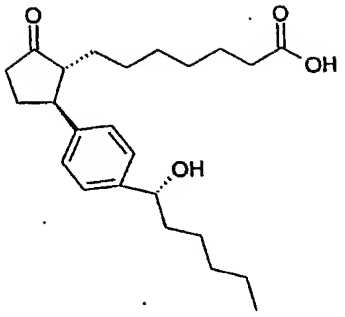
The four major stereoisomers have the following structures:



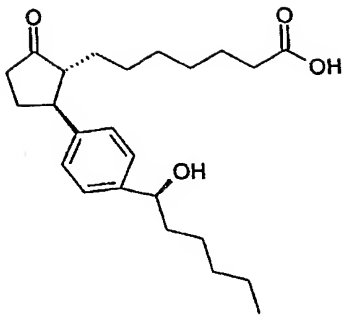
7-{(1S)-2-[(4*R*)-4-((1*R*)-1-Hydroxy-hexyl)-phenyl]-5-oxo-cyclopentyl}-heptanoic acid [SRR]



7-{(1S)-2-[(4*R*)-4-((1S)-1-Hydroxy-hexyl)-phenyl]-5-oxo-cyclopentyl}-heptanoic acid [SRS]



7-{(1*R*)-2-[(4*S*)-4-((1*R*)-1-Hydroxy-hexyl) -phenyl]-5-oxo-cyclopentyl}-heptanoic acid [RSR]



7-{(1*R*)-2-[(4*S*)-4-((1*S*)-1-Hydroxy-hexyl) -phenyl]-5-oxo-cyclopentyl}-heptanoic acid [RSS]

The present applicants have been able to separate the four major stereoisomers from each other and have determined their relative activities. However, initial attempts to separate these stereoisomers were not successful.

Attempts were carried out on a mixture of all the stereoisomers in their acid form using chiral HPLC using a variety of commercially available stationary phases, but these were unsuccessful.

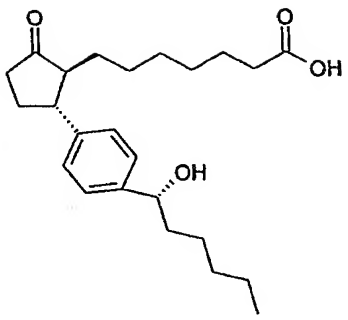
Many attempts at separation were carried out on the two mixtures of esters produced in example 1 below, using chiral HPLC on a variety of commercially available stationary phases and mobile phases, but at best this method was successful on an analytical level and separation was not possible on a preparative scale.

Finally, however, attempts to separate the stereoisomers as esters was successful as is described below in Example 2.

Summary of the invention

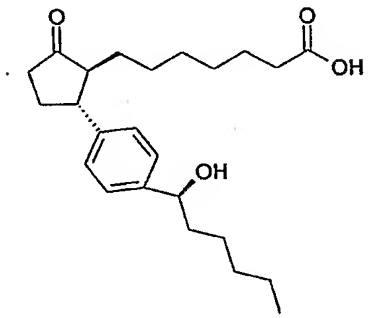
In a first aspect, the present invention provides a compound selected from one of the following:

(i)



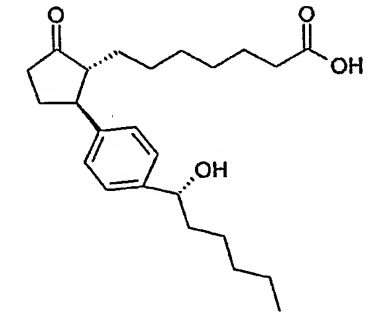
7-{(1*S*)-2-[(4*R*)-4-((1*R*)-1-Hydroxy-hexyl)
-phenyl]-5-oxo-cyclopentyl}-heptanoic acid
[SRR]

(ii)



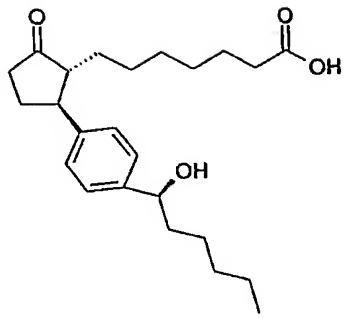
7-{(1*S*)-2-[(4*R*)-4-((1*S*)-1-Hydroxy-hexyl)
-phenyl]-5-oxo-cyclopentyl}-heptanoic acid
[SRS]

(iii)



7-{(1*R*)-2-[(4*S*)-4-((1*R*)-1-Hydroxy-hexyl)
-phenyl]-5-oxo-cyclopentyl}-heptanoic acid
[RSR] ; or

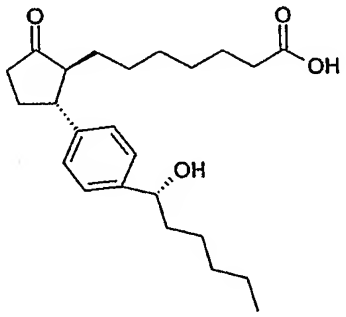
(iv)



7-{(1*R*)-2-[(4*S*)-4-((1*S*)-1-Hydroxy-hexyl)-phenyl]-5-oxo-cyclopentyl}-heptanoic acid [RSS]

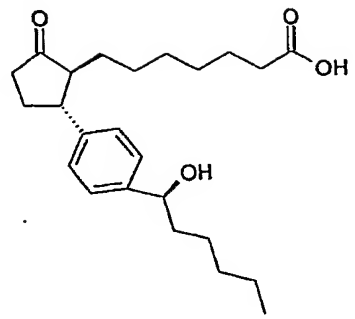
In a second aspect, the present invention provides *trans*-2-[4-(1-hydroxy-hexyl)-phenyl]-5-oxo-cyclopentaneheptanoic acid, of which at least 90% by weight is selected from one of the following forms:

(i)



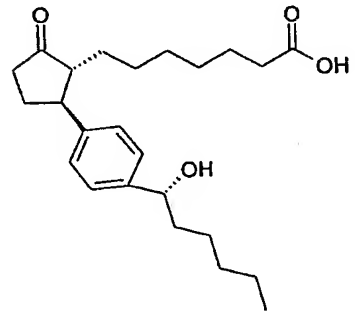
7-{(1*S*)-2-[(4*R*)-4-((1*R*)-1-Hydroxy-hexyl)-phenyl]-5-oxo-cyclopentyl}-heptanoic acid [SRR]

(ii)



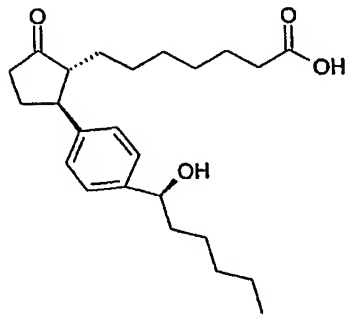
7-{(1*S*)-2-[(4*R*)-4-((1*S*)-1-Hydroxy-hexyl)-phenyl]-5-oxo-cyclopentyl}-heptanoic acid [SRS]

(iii)



7-{(1*R*)-2-[(4*S*)-4-((1*R*)-1-Hydroxy-hexyl)-phenyl]-5-oxo-cyclopentyl}-heptanoic acid [RSR] ; or

(iv)



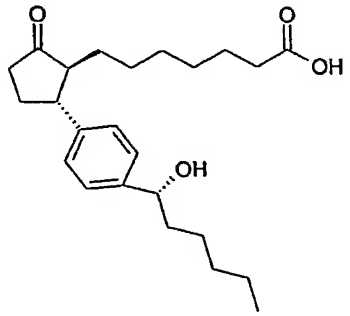
7-{(1*R*)-2-[(4*S*)-4-((1*S*)-1-Hydroxy-hexyl)-phenyl]-5-oxo-cyclopentyl}-heptanoic acid [RSS]

It is preferred that at least 95, 97, 99, 99.5 or 99.9% by weight of the *trans*-2-[4-(1-hydroxy-hexyl)-phenyl]-5-oxo-

cyclopentaneheptanoic acid is in one of the four forms shown.

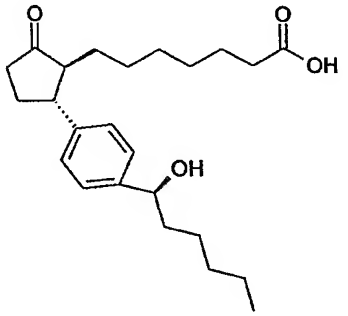
In a third aspect, the present invention provides 2-[4-(1-hydroxy-hexyl)-phenyl]-5-oxo-cyclopentaneheptanoic acid, of which at least 80% by weight is in one of the following forms:

(i)



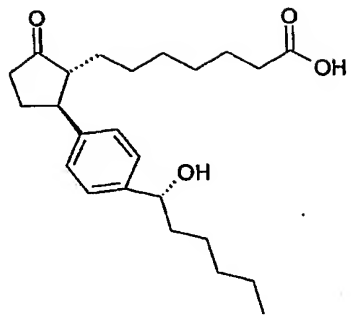
7-{(1*S*)-2-[(4*R*)-4-((1*R*)-1-Hydroxy-hexyl)-phenyl]-5-oxo-cyclopentyl}-heptanoic acid [SRR]

(ii)



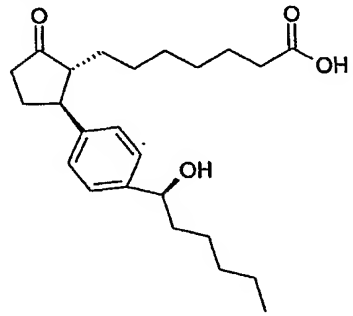
7-{(1*S*)-2-[(4*R*)-4-((1*S*)-1-Hydroxy-hexyl)-phenyl]-5-oxo-cyclopentyl}-heptanoic acid [SRS]

(iii)



7-{(1*R*)-2-[(4*S*)-4-((1*R*)-1-Hydroxy-hexyl)-phenyl]-5-oxo-cyclopentyl}-heptanoic acid [RSR] ; or

(iv)



7-{(1*R*)-2-[(4*S*)-4-((1*S*)-1-Hydroxy-hexyl)-phenyl]-5-oxo-cyclopentyl}-heptanoic acid [RSS]

It is preferred that at least 90, 95, 97, 99, 99.5 or 99.9% by weight of the 2-[4-(1-hydroxy-hexyl)-phenyl]-5-oxo-cyclopentaneheptanoic acid is in one of the four forms shown.

The above three aspects also relate to salts, solvates, chemically protected forms and prodrugs of the compounds described.

A fourth aspect of the invention provides a method of making a compound, comprising the following steps:

(a) asymmetrically reducing 1-(4-bromophenyl)hexan-1-one

with (-)-DIP chloride to produce *(S)*-1-(4-bromophenyl)hexan-1-ol;

(b) converting the *(S)*-1-(4-bromophenyl)hexan-1-ol into *(S)*-1-(4-bromophenyl)-1-(*tert*-butyldimethylsilyloxy)hexane;

(c) treating the *(S)*-1-(4-bromophenyl)-1-(*tert*-butyldimethylsilyloxy)hexane with tert-butyllithium, followed by 1:2 pentynyl copper:hexamethylphosphorus triamide, followed by condensation with 2-(6-carbomethoxyhexyl)cyclopent-2-en-1-one to produce a diastereomeric mixture of *trans*- and *cis*- 2-(6-carbomethoxyhexyl)-3-[4-1(1-(*S*)-(tert-butyldimethylsilyoxy)hexyl)phenyl]cyclopentanone;

(d) deprotecting the t-butyldimethyl silyl group to give a diastereomeric mixture of *trans*- and *cis*- 2-(6-carbomethoxyhexyl)-3-[4-1(1-(*S*)-hydroxyhexyl)phenyl]cyclopentanone;

(e) subjecting the diastereomeric mixture to HPLC on a chiral stationary phase, which is amylose tris(3,5-dimethylphenyl-carbamate adsorbed on a macroporous silica gel support that had been treated with 3-aminopropyl triethoxysilane in benzene, using a mobile phase of 100% ethanol;

(f) substantially isolating a single stereoisomer, being a fraction in the eluent.

A fifth aspect of the invention provides a method of making a compound, comprising the following steps:

(a) asymmetrically reducing 1-(4-bromophenyl)hexan-1-one with (+)-DIP chloride to produce *(R)*-1-(4-bromophenyl)hexan-1-ol;

(b) converting the *(R)*-1-(4-bromophenyl)hexan-1-ol into *(R)*- 1-(4-bromophenyl)-1-(*tert*-butyldimethylsilyloxy)hexane;

(c) treating the *(R)*- 1-(4-bromophenyl)-1-(*tert*-butyldimethylsilyloxy)hexane with tert-butyllithium,

followed by 1:2 pentynyl copper:hexamethylphosphorus triamide, followed by condensation with 2-(6-carbomethoxyhexyl)cyclopent-2-en-1-one to produce a diastereomeric mixture of *trans*- and *cis*- 2-(6-carbomethoxyhexyl)-3-[4-1(1-(*R*)-(tert-butyldimethylsilyoxy)hexyl)phenyl]cyclopentanone;

(d) deprotecting the t-butyldimethyl silyl group to give a diastereomeric mixture of *trans*- and *cis*- 2-(6-carbomethoxyhexyl)-3-[4-1(1-(*R*)-hydroxyhexyl)phenyl]cyclopentanone;

(e) subjecting the diastereomeric mixture to HPLC on a chiral stationary phase, which is amylose tris(3,5-dimethylphenyl-carbamate adsorbed on a macroporous silica gel support that had been treated with 3-aminopropyl triethoxysilane in benzene, using a mobile phase of 100% ethanol;

(f) substantially isolating a single stereoisomer, being a fraction in the eluent.

In the fourth and fifth aspects, the term "substantially" means that the compound produced is at least 90% by weight of a single stereoisomer of a compound. Preferably the compound produced is 95, 97, 99, 99.5 or 99.9% by weight of a single stereoisomer of a compound.

A sixth aspect of the present invention provides a compound obtainable by or obtained by the methods of the fourth or fifth aspects. A seventh aspect of the invention provides a method of making a compound according to the first, second or third aspect of the invention, comprising one or more steps as described in the general synthesis section below.

An eighth aspect of the present invention provides a compound of the first to third aspects, or a compound made

(or obtainable) by the methods of the fourth, fifth or seventh aspects, or a pharmaceutically acceptable salt thereof for use in a method of therapy.

A ninth aspect of the present invention provides a pharmaceutical composition comprising a compound of the first to third aspects, or a compound made by the methods of the fourth to sixth aspects, or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier or diluent.

A further aspect of the present invention provides the use of a compound of the first to third aspects, or a compound made by (or obtainable by) the methods of the fourth, fifth or seventh aspects, or a pharmaceutically acceptable salt thereof in the preparation of a medicament for the treatment of a condition alleviated by agonism of an EP_2 receptor.

Another aspect of the present invention provides a method of treating a condition which can be alleviated by agonism of an EP_2 receptor, which method comprises administering to a patient in need of treatment an effective amount of a compound of the first to third aspects, or a compound made by (or obtainable by) the methods of the fourth, fifth or seventh aspects, or a pharmaceutically acceptable salt thereof.

Conditions which can be alleviated by agonism of an EP_2 receptor are discussed above, and particularly include dysmenorrhoea, pre-term labour, glaucoma, ocular hypertension, immune disorders, osteoporosis, asthma, allergy, bone disease, fracture repair, male sexual dysfunction, female sexual dysfunction, periodontal disease, gastric ulcer and renal disease.

EP receptor agonists are also know to inhibit IL-2 production, although the EP receptor involved has not been previously defined. The present inventors have discovered that EP_2 agonists inhibit IL-2 production, which has been shown to be useful in treating psoriasis (Salim, A. & Emerson, R., *Curr. Opin. Investig. Drugs*, **2(11)**, 1546-8 (2001)). Therefore, a further condition which can be alleviated by agonism of an EP_2 receptor is psoriasis.

Furthermore, aspects of the present invention relate to the use of EP_2 agonists to treat conditions ameliorated by the inhibtion of IL-2 production and the use of an EP_2 in the thereof in the preparation of a medicament for the treatment of a condition alleviated by inhibition of IL-2 production.

The present invention also provides methods of agonizing EP_2 receptors and/or inhibting the production of IL-2, *in vitro* or *in vivo*, comprising contacting a cell with an effective amount of a compound of the first to third aspects, or a compound made (or obtainable) by the methods of the fourth, fifth or seventh aspects.

In some embodiments, the compounds described above may be selective as against agonism of the other three EP receptors, i.e. EP_1, EP_3 and EP_4. This selectivity allows for targeting of the effect of the compounds of the invention, with possible benefits in the treatment of certain conditions.

The invention will be described with reference to the attached figures, in which:

Figure 1 shows the variation in percentage of $[^3H]PGE_2$ displaced with concentration of five test compounds in an assay of binding ability to human EP receptors;

Figure 2 shows the variation in concentration of cAMP with concentration of five test compounds in an assay of cyclase production;

Figure 3 shows the effect on human myometrial activity of AH13205;

Figure 4 shows the variation in % inhibition of electrical field stimulation (EFS) induced contractions with concentrations of AH13205 and delivery vehicle or delivery vehicle alone in an assay of human myometrial activity;

Figure 5 shows the variation in % of control electrical field stimulation (EFS) induced contractions with concentrations of three test compounds in an assay of human myometrial activity;

Figure 6 shows the variation in IL-2 production with concentration of 4 test compounds in a lymphocyte assay;

Figure 7 shows the variation of TNFα production in response to 3 test compounds in a monocyte assay.

Definitions

Includes Other Forms

Unless otherwise specified, included in the above are the well known ionic, salt, solvate, and protected forms of these substituents. For example, a reference to carboxylic acid (-COOH) also includes the anionic (carboxylate) form ($-COO^-$), a salt or solvate thereof, as well as conventional

protected forms. Similarly, a reference to a hydroxyl group also includes the anionic form ($-O^-$), a salt or solvate thereof, as well as conventional protected forms of a hydroxyl group.

Salts, Solvates and Protected Forms

It may be convenient or desirable to prepare, purify, and/or handle a corresponding salt of the active compound, for example, a pharmaceutically-acceptable salt. Examples of pharmaceutically acceptable salts are discussed in Berge, *et al.*, *J. Pharm. Sci.*, **66**, 1-19 (1977).

For example, if the compound is anionic, or has a functional group which may be anionic (e.g. -COOH may be $-COO^-$), then a salt may be formed with a suitable cation. Examples of suitable inorganic cations include, but are not limited to, alkali metal ions such as Na^+ and K^+, alkaline earth cations such as Ca^{2+} and Mg^{2+}, and other cations such as Al^{+3}. Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e. NH_4^+) and substituted ammonium ions (e.g. NH_3R^+, $NH_2R_2^+$, NHR_3^+, NR_4^+). Examples of some suitable substituted ammonium ions are those derived from: ethylamine, diethylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary ammonium ion is $N(CH_3)_4^+$.

It may be convenient or desirable to prepare, purify, and/or handle a corresponding solvate of the active compound. The term "solvate" is used herein in the conventional sense to refer to a complex of solute (e.g., active compound, salt of active compound) and solvent. If the solvent is water, the

solvate may be conveniently referred to as a hydrate, for example, a mono-hydrate, a di-hydrate, a tri-hydrate, etc.

It may be convenient or desirable to prepare, purify, and/or handle the active compound in a chemically protected form. The term "chemically protected form" is used herein in the conventional chemical sense and pertains to a compound in which one or more reactive functional groups are protected from undesirable chemical reactions under specified conditions (e.g. pH, temperature, radiation, solvent, and the like). In practice, well known chemical methods are employed to reversibly render unreactive a functional group, which otherwise would be reactive, under specified conditions. In a chemically protected form, one or more reactive functional groups are in the form of a protected or protecting group (also known as a masked or masking group or a blocked or blocking group). By protecting a reactive functional group, reactions involving other unprotected reactive functional groups can be performed, without affecting the protected group; the protecting group may be removed, usually in a subsequent step, without substantially affecting the remainder of the molecule. See, for example, Protective Groups in Organic Synthesis (T. Green and P. Wuts; 3rd Edition; John Wiley and Sons, 1999).

A wide variety of such "protecting", "blocking", or "masking" methods are widely used and well known in organic synthesis. For example, a compound which has two nonequivalent reactive functional groups, both of which would be reactive under specified conditions, may be derivatized to render one of the functional groups "protected," and therefore unreactive, under the specified conditions; so protected, the compound may be used as a reactant which has effectively only one reactive functional

group. After the desired reaction (involving the other functional group) is complete, the protected group may be "deprotected" to return it to its original functionality.

For example, a hydroxy group may be protected as an ether (-OR) or an ester (-OC(=O)R), for example, as: a t-butyl ether; a benzyl, benzhydryl (diphenylmethyl), or trityl (triphenylmethyl) ether; a trimethylsilyl or t-butyldimethylsilyl ether; or an acetyl ester (-OC(=O)CH_3, -OAc).

For example, a carboxylic acid group may be protected as an ester for example, as: an C_{1-7} alkyl ester (e.g., a methyl ester; a t-butyl ester); a C_{1-7} haloalkyl ester (e.g., a C_{1-7} trihaloalkyl ester); a triC_{1-7} alkylsilyl-C_{1-7} alkyl ester; or a C_{5-20} aryl-C_{1-7} alkyl ester (e.g. a benzyl ester; a nitrobenzyl ester); or as an amide, for example, as a methyl amide.

Prodrugs

It may be convenient or desirable to prepare, purify, and/or handle the active compound in the form of a prodrug. The term "prodrug," as used herein, pertains to a compound which, when metabolised (e.g., in vivo), yields the desired active compound. Typically, the prodrug is inactive, or less active than the active compound, but may provide advantageous handling, administration, or metabolic properties.

Unless otherwise specified, a reference to a particular compound also include prodrugs thereof.

For example, some prodrugs are esters of the active compound (e.g., a physiologically acceptable metabolically labile

ester). During metabolism, the ester group (-C(=O)OR) is cleaved to yield the active drug. Such esters may be formed by esterification, for example, of any of the carboxylic acid groups (-C(=O)OH) in the parent compound, with, where appropriate, prior protection of any other reactive groups present in the parent compound, followed by deprotection if required.

Examples of such metabolically labile esters include those of the formula -C(=O)OR wherein R is:
C_{1-7} alkyl
(e.g., -Me, -Et, -nPr, -iPr, -nBu, -sBu, -iBu, -tBu);
C_{1-7} aminoalkyl
(e.g., aminoethyl; 2-(N,N-diethylamino)ethyl;
2-(4-morpholino)ethyl); and
acyloxy-C_{1-7}alkyl (e.g., acyloxymethyl; acyloxyethyl;
pivaloyloxymethyl; acetoxymethyl; 1-acetoxyethyl;
1-(1-methoxy-1-methyl)ethyl-carbonxyloxyethyl;
1-(benzoyloxy)ethyl; isopropoxy-carbonyloxymethyl;
1-isopropoxy-carbonyloxyethyl; cyclohexyl-carbonyloxymethyl;
1-cyclohexyl-carbonyloxyethyl; cyclohexyloxy-
carbonyloxymethyl; 1-cyclohexyloxy-carbonyloxyethyl;
(4-tetrahydropyranyloxy) carbonyloxymethyl; 1-(4-
tetrahydropyranyloxy)carbonyloxyethyl; (4-
tetrahydropyranyl)carbonyloxymethyl; and
1-(4-tetrahydropyranyl)carbonyloxyethyl).

Also, some prodrugs are activated enzymatically to yield the active compound, or a compound which, upon further chemical reaction, yields the active compound (for example, as in ADEPT, GDEPT, LIDEPT, etc.). For example, the prodrug may be a sugar derivative or other glycoside conjugate, or may be an amino acid ester derivative.

Treatment and Therapy

The term "treatment", as used herein in the context of treating a condition, pertains generally to treatment and therapy, whether of a human or an animal (e.g. in veterinary applications), in which some desired therapeutic effect is achieved, for example, the inhibition of the progress of the condition, and includes a reduction in the rate of progress, a halt in the rate of progress, amelioration of the condition, and cure of the condition. Treatment as a prophylactic measure (i.e. prophylaxis) is also included.

The term "therapeutically-effective amount", as used herein, pertains to that amount of an active compound, or a material, composition or dosage form comprising an active compound, which is effective for producing some desired therapeutic effect, commensurate with a reasonable benefit/risk ratio, when administered in accordance with a desired treatment regimen. Suitable dose ranges will typically be in the range of from 0.01 to 20 mg/kg/day, preferably from 0.1 to 10 mg/kg/day.

Compositions and their administration

Compositions may be formulated for any suitable route and means of administration. Pharmaceutically acceptable carriers or diluents include those used in formulations suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which

constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

For solid compositions, conventional non-toxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, cellulose, cellulose derivatives, starch, magnesium stearate, sodium saccharin, talcum, glucose, sucrose, magnesium carbonate, and the like may be used. The active compound as defined above may be formulated as suppositories using, for example, polyalkylene glycols, acetylated triglycerides and the like, as the carrier. Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, etc, an active compound as defined above and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, sorbitan monolaurate, triethanolamine oleate, etc. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remington's Pharmaceutical Sciences, 20th edition, pub. Lippincott, Williams & Wilkins, 2000. The composition or formulation to be administered will, in any event, contain a quantity of the active compound(s) in an amount effective to alleviate the symptoms of the subject being treated.

Dosage forms or compositions containing active ingredient in the range of 0.25 to 95% with the balance made up from non-toxic carrier may be prepared.

For oral administration, a pharmaceutically acceptable non-toxic composition is formed by the incorporation of any of the normally employed excipients, such as, for example, pharmaceutical grades of mannitol, lactose, cellulose, cellulose derivatives, sodium crosscarmellose, starch, magnesium stearate, sodium saccharin, talcum, glucose, sucrose, magnesium carbonate, and the like. Such compositions take the form of solutions, suspensions, tablets, pills, capsules, powders, sustained release formulations and the like. Such compositions may contain 1%-95% active ingredient, more preferably 2-50%, most preferably 5-8%.

Parenteral administration is generally characterized by injection, either subcutaneously, intramuscularly or intravenously. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like. In addition, if desired, the pharmaceutical compositions to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate, triethanolamine sodium acetate, etc.

The percentage of active compound contained in such parental compositions is highly dependent on the specific nature thereof, as well as the activity of the compound and the

needs of the subject. However, percentages of active ingredient of 0.1% to 10% in solution are employable, and will be higher if the composition is a solid which will be subsequently diluted to the above percentages. Preferably, the composition will comprise 0.2-2% of the active agent in solution.

Formulations suitable for transdermal administration include gels, pastes, ointments, creams, lotions, and oils, as well as patches, adhesive plasters, bandages, dressings, depots, and reservoirs.

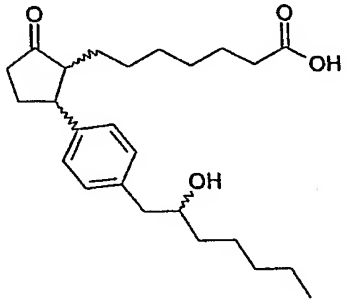
Ointments are typically prepared from the active compound and a paraffinic or a water-miscible ointment base.

Creams are typically prepared from the active compound and an oil-in-water cream base. If desired, the aqueous phase of the cream base may include, for example, at least about 30% w/w of a polyhydric alcohol, i.e., an alcohol having two or more hydroxyl groups such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol and mixtures thereof. The topical formulations may desirably include a compound which enhances absorption or penetration of the active compound through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethylsulfoxide and related analogues.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active compound, such carriers as are known in the art to be appropriate.

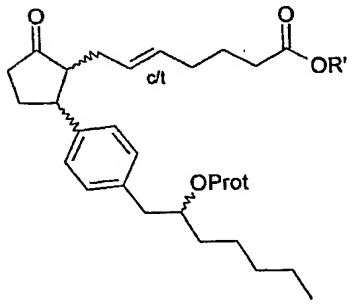
General Synthesis Methods

Compounds of formula 1:



Formula 1

wherein ~ represents a defined stereochemistry at each chiral centre, and the groups on the pentanone are *trans* to one another, may be synthesised from compounds of formula 2:

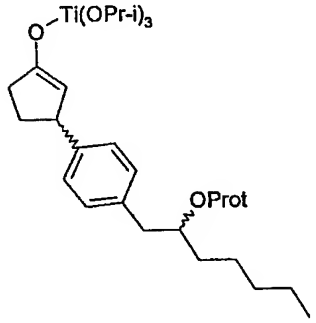


Formula 2

having the same stereochemistry at each chiral centre, wherein R' represents a C_{1-7} alkyl group (a monovalent moiety obtained by removing a hydrogen atom from a carbon atom of a hydrocarbon compound having from 1 to 7 carbon atoms, e.g. methyl (C_1), ethyl (C_2), propyl (C_3), butyl (C_4), pentyl (C_5), hexyl (C_6), heptyl (C_7)) by reduction of the double bond and deprotection of the acid and alcohol using standard techniques e.g. the reduction may be carried out with hydrogen, palladium on charcoal in a solvent such as ethyl acetate at normal temperature and pressure. A particularly preferred alcohol protecting group is a silyl group, such as tert-butyl-di-methyl-silyl (TBDMS), which can be removed,

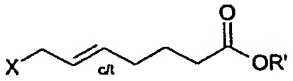
for example, with aqueous acid and a co-solvent, which conditions may also deprotect the acid group. These reactions may be carried out in either order. The double bond may be either in the *cis*- or *trans*- orientation, or a mixture of these.

Compounds of formula 2 may be synthesised by trapping an enolate of formula 3:



Formula 3

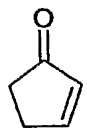
having the same stereochemistry at each of the two chiral centres, with a compound of formula 4:



Formula 4

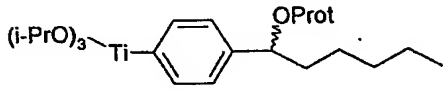
wherein X is a leaving group, such as halide or mesylate, and R′ is as in formula 2, in the presence of a strong base, such as Li O*i*-Pr at room temperature.

The four enolates of formula 3 may be generated from cyclopent-2-enone (formula 5):



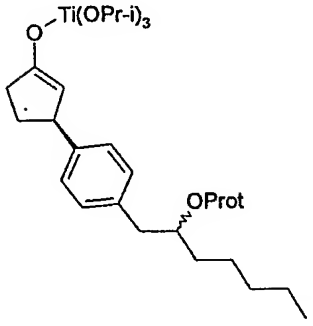
Formula 5

by reacting it with a compound of formula 6:



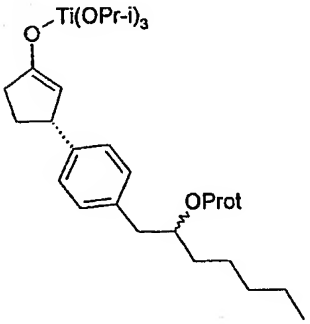
Formula 6

having the same stereochemistry at the chiral centre, in the presence of a transition metal catalyst, preferably Rh(I), in the presence of a chiral ligand, such as BINAP, (2,2'-Bis(diphenylphosphino)-1,1'-binaphthyl). Using *S*-BINAP would yield enolates of formula 3a:



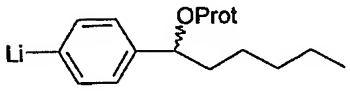
Formula 3a

whereas using *R*-BINAP would yield enolates of formula 3b:



Formula 3b

The compounds of formula 6 may be generated in situ from the reaction of compounds of formula 7:

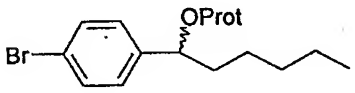


Formula 7

having the same stereochemistry at the chiral centre, with $ClTi(Oi\text{-}Pr)_3$, before the reaction of compounds of formulae 5 and 6. This reaction may be carried generally in accordance with the methods described in Hayashi, T., *et al.*, *JACS*, **124**, 12102-12103 (2002), such as 1.6 equivalents of the compound of formula 6 to the compound of formula 5, with 3% of the catalyst in tetrahydrofuran at 20°C for 1 hour under

an inert atmosphere.

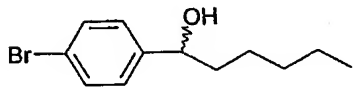
Compounds of formula 7 can be generated from the corresponding bromo compound of formula 8:



Formula 8

with the same stereochemistry at the chiral centre, by treating with an alkyl lithium, in a solvent, for example THF.

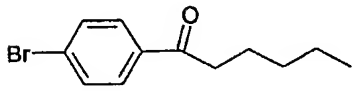
Compounds of formula 8 are made by protecting compounds of formula 9:



Formula 9

with the same stereochemistry at the chiral centre, using standard conditions, which retain the stereochemistry of the chiral centre, e.g. reaction with TBDMSCl or TBDMSOH.

The single stereoisomers of compound 9 can by made from a compound of formula 10:



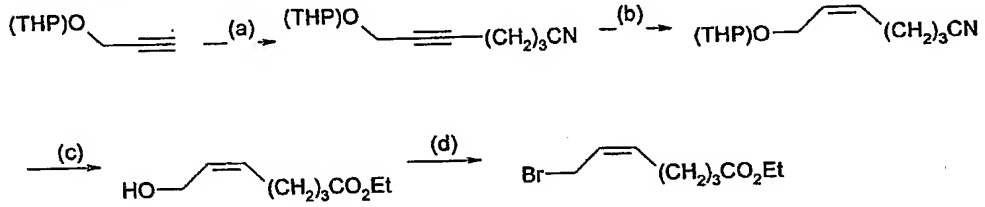
Formula 10

by either enantioselective reduction (e.g. see Brown, H.C., *et al. J. Am. Chem. Soc.*, **110**, 1539-1546 (1988)), or by reduction to the racemate of compound 9 followed by optical resolution.

Compounds of formula 4 are known from the synthesis of natural prostaglandins, e.g. Suzuki, M., *et al.*,

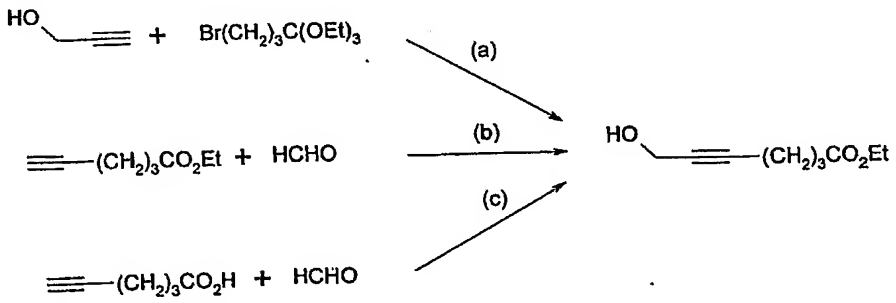
J.Am.Chem.Soc., **107**, 3348-3349 (1985), and may be synthesised by a variety of routes.

One route, based on a route disclosed in Taber, D.F., *et al.*, *J. Org. Chem.*, **62**, 194-198 (1997), is as follows:



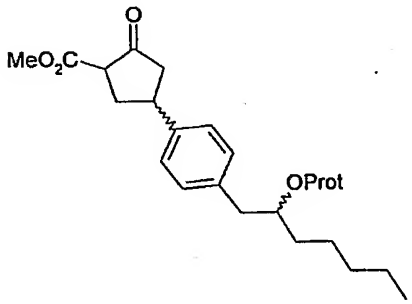
(a) BuLi, $Br(CH_2)_3Br$ (80%); NaCN, DMSO (99%);
(b) $Ni(OAc)_2$, $NaBH_4$ (87%);
(c) Dowex, MeOH (78%); NaOH, EtOH, BF_3 (84%);
(d) CBr_4, Ph_3P (76%)
(34% th.; 57% wt. overall)

Other possible methods use different alkylating agents for the propargyl alcohol (and are illustrated below), but preparation of the alkylating agents require additional step. An example of this is alkylation of the ortho ester of bromobutyrate (Patterson, J.W., *et al.*, *Synthesis*, 1985, 337-338). The ortho ester of 5-hexynoic acid has been reacted with BuLi/formaldehyde to give the same intermediate (Harmann, P, R. and Wissner, A., *Synth.Commun.*, **19**, 1509 (1989)). A further possible route may involve the direct reaction of 5-hexynoic acid (commercially available, Aldrich) with base and formaldehyde.



(a) BuLi
(b) BuLi
(c) base

An alternative route to the four compounds of formula 1 is from compounds of formula 11:

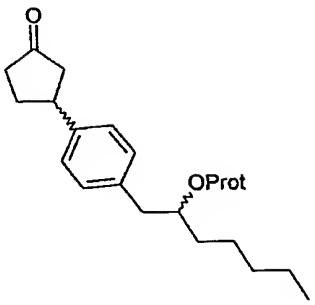


Formula 11

where the chiral centres have the same stereochemistry, by reaction first with sodium hydride and then with strong base, such as potassium amide or butyl lithium, to form the dianion, (Weiler, L., *J.Am.Chem.Soc.*, **92**, 6702-6704 (1970) and see, for example, Modern Synthetic Reactions, 2nd Edition 1972, H.O. House, p. 553), which can then be reacted with haloheptanoate to give the substituted ketoester (Huckin, S.N. and Weiler, L., *Can.J.Chem.*, **52**, 2157 (1974)), which subsequently can be hydrolysed and decarboxylated using standard conditions, e.g. heating with aqueous acid, treating with litium iodide in collidine, treating with sodium cyanide in DMSO (see Modern Synthetic Reactions, 2nd Edition 1972, H.O. House, p. 511-517). The trans arrangment of the groups on the cyclopentanone in the substituted ketoester arises due to steric hindrance.

It may be necessary to replace the haloheptanoate with a more reactive alkylating agent such as the allylic halide of Formula 4, followed by reduction of the double bond.

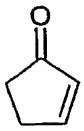
The compounds of formula 11 can be synthesised from compounds of formula 12:



Formula 12

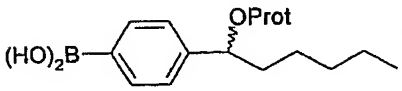
having the same stereochemistry at each of the two chiral centres, by reaction with dimethyl carbonate or methyl chloroformate and a base, such as sodium hydride, in a solvent such as toluene or THF, with mild heating.

The compounds of formula 12 can be synthesised from cyclopent-2-enone (formula 5):



Formula 5

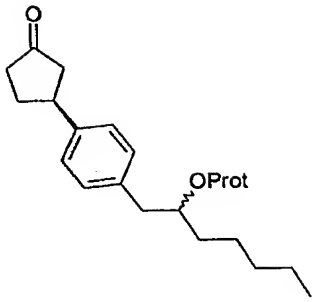
by boronic acid addition (see Takaya, Y., *et al.*, *J.Am.Chem.Soc.*, **120**, 5579-5580 (1998) and Hayashi, T., *Synlett*, **SI**, 879-887 (2001)) of a compound of formula 13:



Formula 13

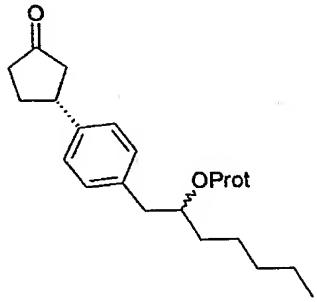
with the same stereochemistry at the chiral centre, in the presence of a transition metal catalyst, preferably Rh(I), in the presence of a chiral ligand, preferably BINAP. Suitable conditions include the use of 3% catalyst and chiral ligand in aqueous dioxane at 60°C for 20 hours.

By analogy with established chemical precedent, use of S-BINAP yields compounds of formula 12a:



Formula 12a

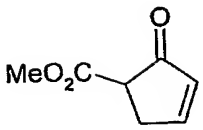
whilst using R-BINAP yields compounds of formula 12b:



Formula 12b

Compounds of formula 13 may be generated from compounds of formula 8, with the same stereochemistry at the chiral centre, by standard techniques. Such techniques include first treatment with a lithium exchange reagent, for example butyl lithium, in a solvent, for example THF, at a suitable temperature (for butyl lithium in THF, -78°C). This is followed by treatment with an appropriate boron reagent, for example $B(O^{i}Pr)_3$ followed by hydrolysis, e.g. by potassium hydroxide (Thompson, W.J. and Gaudino, J., *J.Org.Chem.*, **49**, 5237-5243 (1984)).

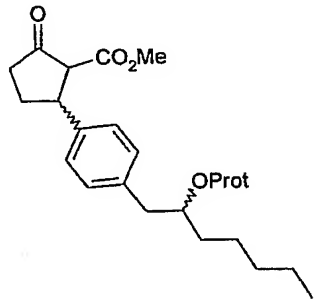
An alternative route from compounds of formula 13 to compounds of formula 11 where the chiral centres have the same stereochemistry is reaction of compounds of formula 13 with the methylcarboxy substituted cyclopent-2-enone of formula 14:



Formula 14

by boronic acid addition, in the presence of a transition metal catalyst, preferably Rh(I), in the presence of a chiral ligand, preferably BINAP. Suitable conditions include the use of 3% catalyst and chiral ligand in aqueous dioxane at 60°C for 20 hours, i.e. similar reaction conditions used for the coupling of compound 5 with compounds of formula 13.

A further alternative route to the four compounds of formula 1 is from compounds of formula 15:

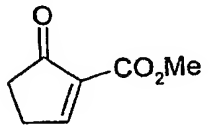


Formula 15

where the chiral centres have the same stereochemistry, by reaction with strong base, such as sodium hydride, e.g. in DMF, to form a monoanion, which can then be reacted with haloheptanoate to give the substituted ketoester, which subsequently can be hydrolysed and decarboxylated using standard conditions, e.g. heating with aqueous acid, treating with litium iodide in collidine, treating with sodium cyanide in DMSO (see Modern Synthetic Reactions, 2nd Edition 1972, H.O. House, p. 511-517). The trans arrangement on the cyclopentanone arises due to steric hindrance.

The compounds of formula 15 can be synthesized by coupling compounds of formula 13, with the same stereochemistry at

the chiral centre, with the methylcarboxy substituted cyclopent-2-enone of formula 16:



Formula 16

(Funk, R.L., *et al.*, *J.Am.Chem.Soc*, **115**, 8849-8850 (1993)) by boronic acid addition, in the presence of a transition metal catalyst, preferably Rh(I), in the presence of a chiral ligand, preferably BINAP. Suitable conditions include the use of 3% catalyst and chiral ligand in aqueous dioxane at 60°C for 20 hours, i.e. similar reaction conditions used for the coupling of compound 5 with compounds of formula 13.

Acronyms

For convenience, many chemical moieties are represented using well known abbreviations, including but not limited to, methyl (Me), ethyl (Et), n-propyl (nPr), iso-propyl (iPr), n-butyl (nBu), sec-butyl (sBu), iso-butyl (iBu), tert-butyl (tBu), n-hexyl (nHex), cyclohexyl (cHex), phenyl (Ph), biphenyl (biPh), benzyl (Bn), naphthyl (naph), methoxy (MeO), ethoxy (EtO), benzoyl (Bz), and acetyl (Ac).

For convenience, many chemical compounds are represented using well known abbreviations, including but not limited to, methanol (MeOH), ethanol (EtOH), iso-propanol (i-PrOH), methyl ethyl ketone (MEK), ether or diethyl ether (Et_2O), acetic acid (AcOH), dichloromethane (methylene chloride, DCM), acetonitrile (ACN), trifluoroacetic acid (TFA), dimethylformamide (DMF), tetrahydrofuran (THF), ethyl acetate (EA) and dimethylsulfoxide (DMSO).

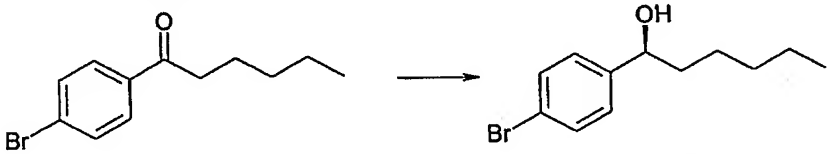
Selectivty

The selectivity of the compound for agonising EP_2 receptors over the other EP receptors (i.e. EP_1, EP_3, EP_4) can be quantified by dividing the Ki for EP_2 (see below) by the Ki for the other EP receptors (see below). The resulting inverse ratio is preferably 10 or more, more preferably 100 or more.

Examples

Example 1: Synthesis of two mixtures each containing 4 stereoisomers of methyl esters of AH13205

(a)(i) Asymmetric reduction of 1-(4-bromophenyl)hexane-1-one with (-)-DIP chloride [B-chlorodiisopinocampheylborane] to (S)-1-(4-Bromophenyl)hexan-1-ol ***(2a)***



1 2a

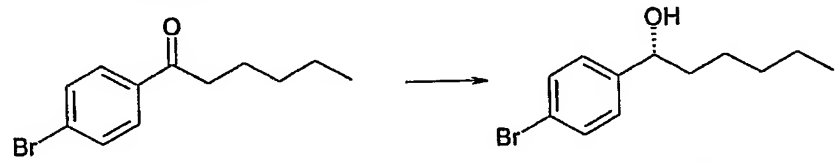
To a solution of (-)-DIP chloride (13.5g) in anhydrous THF (20ml), cooled to -25°C, was added a solution of 1-(4-bromophenyl)hexan-1-one (10g) in anhydrous THF (20ml) over 5-10 minutes keeping the temperature below -20°C. The mixture was kept at -25°C for the next 6 hours then added to a vigorously stirred mixture of diethanolamine (12ml) and triethylamine (10ml) in ether (250ml). The mixture was left stirring overnight, washed with dilute hydrochloric acid, brine, dried over sodium sulphate and evaporated *in vacuo*. Compound **2a** (4.5g; m.p. 70-71°C) was obtained following silica-gel column chromatography of the residue in dichloromethane followed by re-crystallisation from heptane.

$[\alpha]_{24}$ = -26.5 (c = 4.00; $CHCl_3$)

^1H NMR ($CDCl_3$, δ): 0.85 (3H, t); 1.2-1.9 (9H, m); 4.6 (1H, t); 7.15 (2H, d); 7.45 (2H, d).

HPLC (Chiracel OD 250 x 4.6mm, eluant hexane:IPA 99:1, flow rate 0.5ml/min, λ=254nm): 44 minutes, e.e.100%.

(a)(ii) Asymmetric reduction of 1-(4-bromophenyl)hexane-1-one with (+)-DIP chloride [B-chlorodiisopinocampheylborane] to (R)-1-(4-Bromophenyl)hexan-1-ol ***(2b)***



1 2b

Compound **2b** (4g; m.p. 70-71°C) was made from (+)-DIP chloride (13.5g) and 1-(4-bromophenyl)hexan-1-one (10g) by an analogous method to that described in Example 1(a)(i).

$[\alpha]_{24}$ = +27.3 (c= 4.06; $CHCl_3$)

m/z (EIMS) : 256, 258

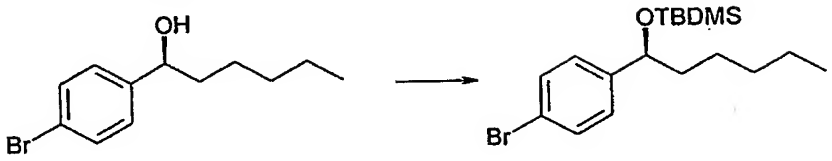
^1H NMR ($CDCl_3$, δ): 0.85 (3H, t); 1.2-1.9 (9H, m); 4.6 (1H, t); 7.15 (2H, d); 7.45 (2H, d).

HPLC (Chiracel OD 250 x 4.6mm, eluant hexane: IPA 99:1, flow rate 0.5ml/min, λ=254nm): 47 minutes, e.e.100%.

The absolute stereochemistry of compounds **2a** and **2b** was assigned by analogy with a literature method for reducing long chain aromatic ketones described by Brown, H.C., *et al.* *J. Am. Chem. Soc.*, **110**, 1539-1546 (1998). The alcohols were shown to be essentially homochiral by chiral HPLC.

(b)(i) Synthesis of (S)-1-(4-Bromophenyl)-1-(tert-

butyldimethylsilyloxy)hexane ***(3a)***



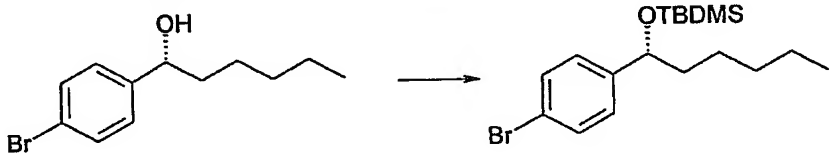
2a 3a

A mixture of (*S*)-1-(4-bromophenyl)hexan-1-ol (**2a**)(10g), *tert*-butyldimethylsilyl chloride (7g) and imidazole (3.7g) were stirred in anhydrous dimethylformamide (100ml) for 16 hours. The mixture was partitioned between petroleum ether and water and the layers separated. The organic layer was washed with water, brine, dried over sodium sulphate and evaporated *in vacuo*. Compound **3a** (14.5g) was obtained as an oil following column chromatography of the residue in petroleum ether.

m/z (EIMS): 370, 372

^{1}H NMR ($CDCl_3$, δ): -0.2 (3H, s); 0.0 (3H, s); 0.85 (9H, s); 0.85 (3H, t); 1.25 (6H, m) 1.55 (2H, m); 4.6 (1H, m); 7.1 (2H, d); 7.4 (2H, d).

(b)(ii) Synthesis of (R)-1-(4-Bromophenyl)-1-(tert-butyldimethylsilyloxy)hexane ***(3b)***



2b 3b

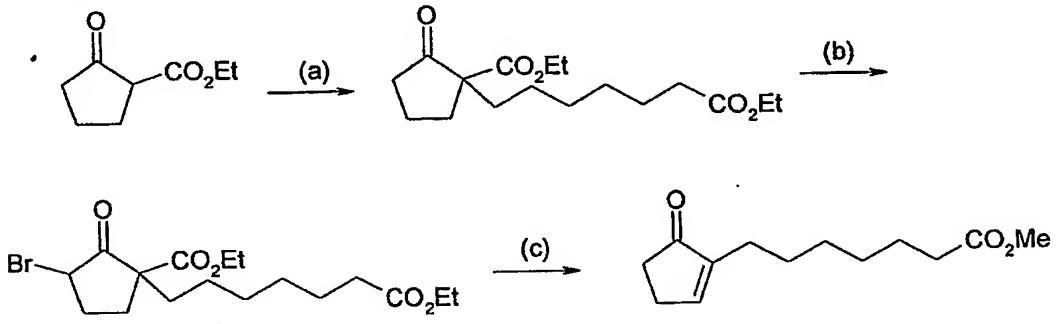
Compound **3b**(18.5g) was made from (*R*)-1-(4-bromophenyl)hexan-1-ol (**2b**)(12.5g) by an analogous method to that described in Example 1(b)(i).

m/z (EIMS): 370, 372

^{1}H NMR ($CDCl_3$, δ): -0.2 (3H, s); 0.0 (3H, s); 0.85 (9H, s);

0.85 (3H, t); 1.25 (6H, m) 1.55 (2H, m); 4.6 (1H, m); 7.1 (2H, d); 7.4 (2H, d).

(c) Synthesis of 2-(6-carbomethoxyhexyl)cyclopent-2-en-1-one (5)

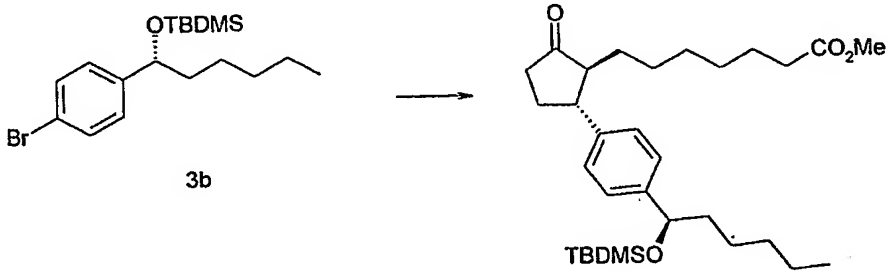


5

(a) ethyl 7-bromoheptanoate, sodium hydride
(b) bromine
(c) (i) acid; (ii) esterify

This known compound, which is commercially available, was prepared in three steps from ethyl 2-oxocyclopentane carboxylate by the methods of Bagli, J. *et al.*, *J. Org. Chem.*, **1972**, 37, 2132-2138 and Bernady, K.F., *J.Org. Chem.*, **1980** 45, 4702-4715.

(d)(i) Synthesis of 2-(6-Carbomethoxyhexyl)-3-[4-(1-(R)-(tert-butyldimethylsilyloxy) hexyl)phenyl]cyclopentanone diastereomers (circa 3:1 trans:cis mixture) (6b)



3b

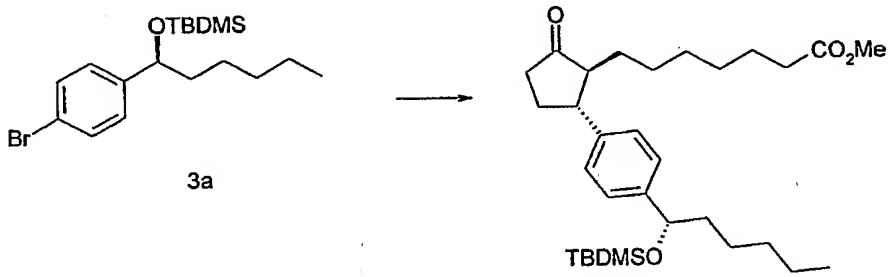
& its diastereoisomer
+ ~30% *cis*-isomer (6b)

To a solution of (*R*)-1-(4-bromophenyl)-1-(*tert*-butyldimethylsilyloxy)hexane (**3b**)(7.2g) in anhydrous diethyl ether (100ml) was added tert-butyllithium (1.5M in hexanes; 28ml) dropwise at -78°C, not allowing the temperature to rise above -60°C. The mixture was left at -78°C for a further 3 hours. A slurry of copper (1) pentyne (2.5g) in anhydrous diethyl ether (56ml) was treated with hexamethylphosphorous triamide (8ml) and the mixture stirred at room temperature for several minutes to form a solution. This freshly prepared solution was now added dropwise to the aryllithium solution at -78°C and left for a further hour at -78°C, whereupon a solution of 2-(6-carbomethoxyhexyl)cyclopent-2-en-1-one (**5**) (4g) in anhydrous diethyl ether (40ml) was added. The reaction mixture was held at -78°C for 15 minutes then at -25°C to -10°C for a further hour. The cold mixture was partitioned quickly between dilute hydrochloric acid and ether, the organic layer separated, washed with brine, dried over sodium sulphate and evaporated *in vacuo*. Compounds **6b**(7.2g) were obtained as a *circa* 3:1 mixture of *trans*:*cis* isomers following silica-gel column chromatography of the residue in 2:1 dichloromethane:petroleum ether then 3:17 ethyl acetate:petroleum ether.

^{1}H NMR ($CDCl_3$, δ) - *trans*-diastereomers: -0.25 (3H, s); 0.0 (3H, s); 0.85 (9H, s); 0.8-2.0 (22H, m); 2.2-2.6 (6H, m); 2.95 (1H, m); 3.65 (3H, s); 4.6 (1H, m); 7.15 (2H, d); 7.25 (2H, d).

^{1}H NMR ($CDCl_3$, δ) - *cis*-diastereomers: -0.27 (3H, s); -0.02 (3H, s); 0.85 (9H, s); 0.8-2.0 (22H, m); 2.2-2.6 (6H, m); 3.55 (1H, m); 3.65 (3H, s); 4.6 (1H, m); 7.0 (2H, d); 7.15 (2H, d).

(d)(ii) 2-(6-Carbomethoxyhexyl)-3-[4-(1-(S)-(tert-butyldimethylsilyloxy)hexyl)phenyl]cyclopentanone diastereomers (circa 3:1 trans:cis mixture) ***(6a)***



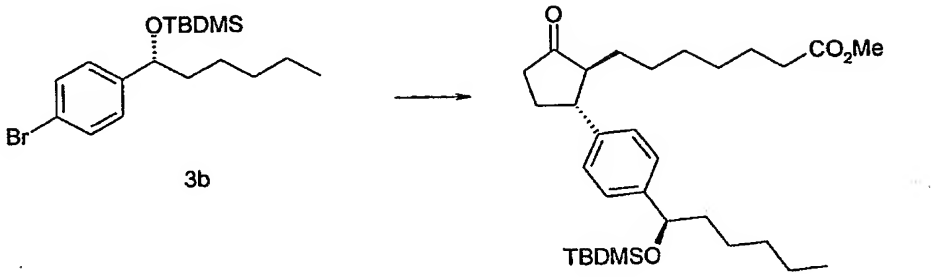
The title compound and its diastereoisomer, and about 30% of their *cis*-isomers (**6a**), (7.3g) were made from (*S*)-1-(4-bromophenyl)-1-(*tert*-butyldimethylsilyloxy)hexane (**3a**)(7.2g) and 2-(6-carbomethoxyhexyl)cyclopent-2-en-1-one (**5**)(4g) by an analogous method to that described in Example 1(d)(i).

^{1}H NMR ($CDCl_3$, δ) - *trans* diastereomers : -0.25 (3H, s); 0.0 (3H, s); 0.85 (9H, s); 0.8-2.0 (22H, m); 2.2-2.6 (6H, m); 2.95 (1H, m); 3.65 (3H, s); 4.6 (1H, m); 7.15 (2H, d); 7.25 (2H, d).

^{1}H NMR ($CDCl_3$, δ) - *cis* diastereomers : -0.27 (3H, s); -0.02 (3H, s); 0.85 (9H, s); 0.8-2.0 (22H, m); 2.2-2.6 (6H, m); 3.55 (1H, m); 3.65 (3H, s); 4.6 (1H, m); 7.0 (2H, d); 7.15 (2H, d).

(d)(iii) *Alternative synthesis of 2-(6-Carbomethoxyhexyl)-3-[4-(1-(R)-(tert-butyldimethylsilyloxy)*

hexyl)phenyl]cyclopentanone diastereomers (circa 3:1 trans:cis mixture) ***(6b)***



6b & its diastereoisomer
+ ~30% *cis*-isomer

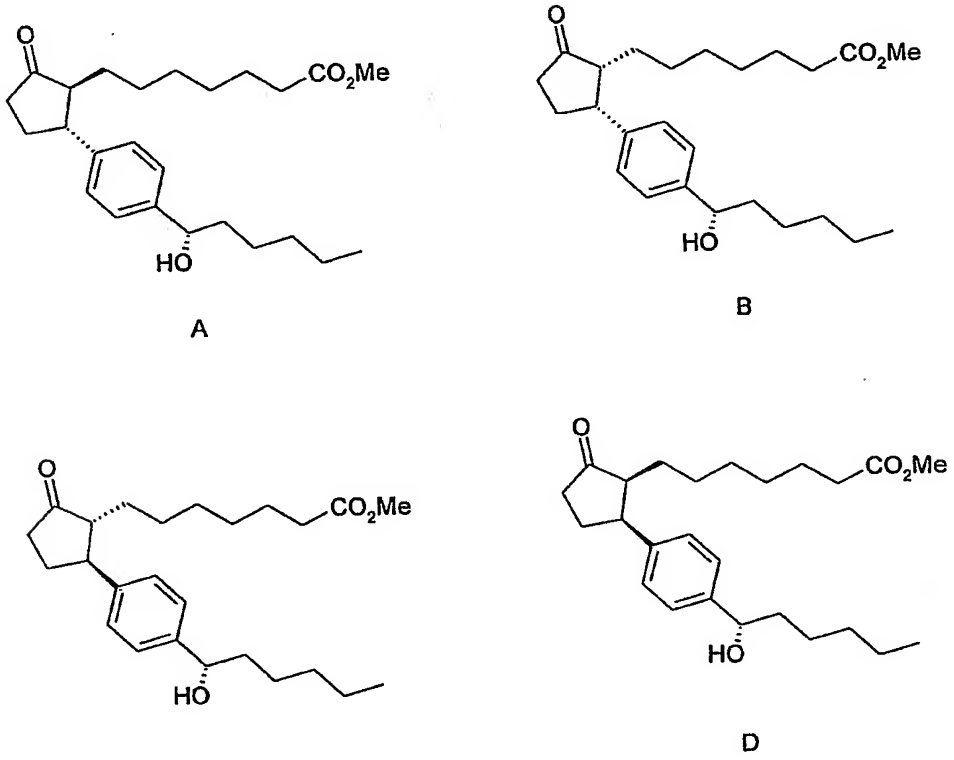
A mixture of (*R*)-1-(4-bromophenyl)-1-(*tert*-butyldimethylsilyloxy)hexane (**3b**)(0.8g), magnesium turnings (0.11g), a crystal of iodine and 1,2-dibromoethane (5µl) in anhydrous THF (4ml) were boiled to reflux to initiate reaction, then kept at 35°C for 2 hours to form the Grignard solution. Lithium chloride (0.125g) and copper (1) bromide.dimethyl sulphide complex (0.61g) were stirred in anhydrous THF (4.5ml) for a few minutes then cooled to -78°C whereupon the Grignard solution was added dropwise. The resulting mixture was left for 5 minutes at -78°C then trimethylsilyl chloride (0.38ml) was added followed by a solution of 2-(6-carbomethoxyhexyl)cyclopent-2-en-1-one (0.18g) in anhydrous THF (1.5ml). The mixture was kept at -78°C for 15 minutes, at 0°C for 30 minutes then allowed to warm up to room temperature for an hour. The mixture was re-cooled to -20°C whereupon dilute hydrochloric acid (4ml) was added and the mixture stirred vigorously for two minutes. The cold mixture was partitioned between petroleum ether and saturated ammonium chloride solution and the layers separated. The organic layer was washed with brine, dried over sodium sulphate and evaporated *in vacuo*. Compounds **6b**

(0.20g) were isolated as a mixture of *cis* and *trans* isomers following silica-gel column chromatography of the residue in 4:1 petroleum ether:ethyl acetate.

^{1}H NMR ($CDCl_3$, δ) - *trans* diastereomers : -0.25 (3H, s); 0.0 (3H, s); 0.85 (9H, s); 0.8-2.0 (22H, m); 2.2-2.6 (6H, m); 2.95 (1H, m); 3.65 (3H, s); 4.6 (1H, m); 7.15 (2H, d); 7.25 (2H, d).

^{1}H NMR ($CDCl_3$, δ) - *cis* diastereomers: -0.27 (3H, s); -0.02 (3H, s); 0.85 (9H, s); 0.8-2.0 (22H, m); 2.2-2.6 (6H, m); 3.55 (1H, m); 3.65 (3H, s); 4.6 (1H, m); 7.0 (2H, d); 7.15 (2H, d).

*(e)(i) Synthesis of cis- and trans- 2-(6-Carbomethoxyhexyl)-3-[4-(1-(S)-hydroxyhexyl)phenyl]cyclopentanone diastereomers (7a) (**Mixture 1**)*



7a (MIXTURE 1)

A: 7-{(1*S*)-2-[(4*R*)-4-((1*S*)-1-Hydroxy-hexyl)-phenyl]-5-oxo-cyclopentyl}-heptanoic acid methyl ester [SRS]

B: 7-{(1*R*)-2-[(4*R*)-4-((1*S*)-1-Hydroxy-hexyl)-phenyl]-5-oxo-cyclopentyl}-heptanoic acid methyl ester [RRS]

C: 7-{(1*R*)-2-[(4*S*)-4-((1*S*)-1-Hydroxy-hexyl)-phenyl]-5-oxo-cyclopentyl}-heptanoic acid methyl ester [RSS]

D: 7-{(1*S*)-2-[(4*S*)-4-((1*S*)-1-Hydroxy-hexyl)-phenyl]-5-oxo-cyclopentyl}-heptanoic acid methyl ester [SSS]

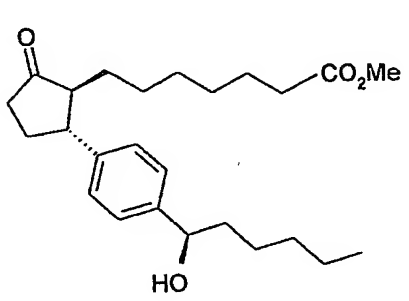
2-(6-Carbomethoxyhexyl)-3-[4-(1-(*S*)-(*tert*-butyldimethylsilyloxy)hexyl)phenyl]cyclopentanone

diastereomers (**6a**)(0.2g; of *circa* 3:1 *trans*:*cis* composition) was stirred in a mixture of THF (3.5ml) and dilute hydrochloric acid (2M; 1ml) for 20 hours at 25°C. The reaction mixture was added to brine and extracted twice with dichloromethane. The combined organic layers were dried over sodium sulphate and evaporated *in vacuo*. 7a (Mixture 1) (0.095g) was obtained as an oil (of *circa* 95:5 *trans*:*cis* composition) following silica-gel column chromatography of the residue in 3:1 petroleum ether:ethyl acetate then 200:3 dichloromethane:methanol.

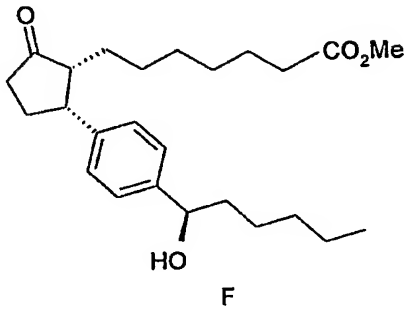
m/z (EIMS): 402

^{1}H NMR ($CDCl_3$, δ) - *trans* diastereomers only: 0.8-2.0 (23H, m); 2.2-2.6 (6H, m); 2.95 (1H, m); 3.65 (3H, s); 4.65 (1H, t); 7.25 (2H, d); 7.35 (2H, d).

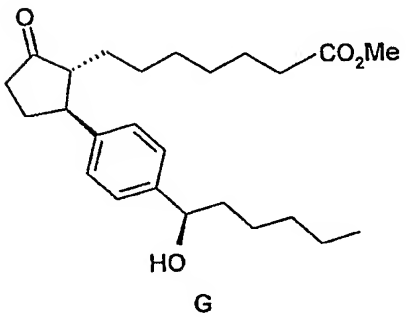
(e)(ii) Synthesis of cis- and trans- 2-(6-Carbomethoxyhexyl)-3-[4-(1-(R)-hydroxyhexyl)phenyl]cyclopentanone diastereomers ***(7b)*** *(Mixture 2)*



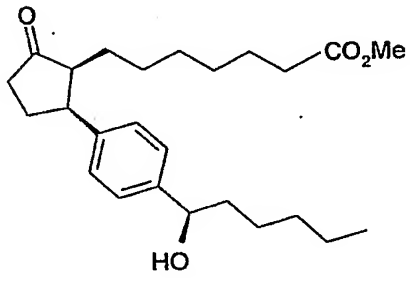
E



F



G



H

7b (MIXTURE 2)

E: 7-{(1*S*)-2-[(4*R*)-4-((1*R*)-1-Hydroxy-hexyl)-phenyl]-5-oxo-cyclopentyl}-heptanoic acid methyl ester [SRR]

F: 7-{(1*R*)-2-[(4*R*)-4-((1*R*)-1-Hydroxy-hexyl)-phenyl]-5-oxo-cyclopentyl}-heptanoic acid methyl ester [RRR]

G: 7-{(1*R*)-2-[(4*S*)-4-((1*R*)-1-Hydroxy-hexyl)-phenyl]-5-oxo-cyclopentyl}-heptanoic acid methyl ester [RSR]

H: 7-{(1*S*)-2-[(4*S*)-4-((1*R*)-1-Hydroxy-hexyl)-phenyl]-5-oxo-cyclopentyl}-heptanoic acid methyl ester [SSR]

Compounds **7b**(0.15g; of *circa* 95:5 *trans*:*cis* composition)) were made from 2-(6-carbomethoxyhexyl)-3-[4-(1-(*R*)-(*tert*-butyldimethylsilyloxy)hexyl)phenyl]cyclopentanone

diastereomers (0.3g; of *circa* 3:1 *trans*:*cis* composition) (**6b**) by an analogous method to that described in Example 1(e)(ii).

m/z (EIMS): 402

^{1}H NMR ($CDCl_3$, δ) - *trans* isomer only: 0.8-2.0 (23H, m); 2.2-2.6 (6H, m); 2.95 (1H, m); 3.65 (3H, s); 4.65 (1H, t); 7.25 (2H, d); 7.35 (2H, d).

Example 2: Separation of *trans*-2-[4-(1-hydroxyhexyl)phenyl]-5-oxo-cyclopentaneheptanoic acid methyl ester diastereomers

HPLC of a 90 mg sample of either ester mixture 1 or 2 on a chiral stationary phase (ChiralPak AD, Daicel Chemical Industries, Japan) using a mobile phase of 100% ethanol afforded complete separation on a column of 25 cm in length by 2 cm internal diameter, in about an hour. A 1g sample of either mixture was separated in eleven consecutive 90 mg runs (flow rate 4ml/min; detection 230 nm). The recovered esters were then hydrolysed to the acids as follows. Methyl ester (0.45g) in 4:1 v/v tetrahydrofuran in water (40ml) was treated with 1M lithium hydroxide in water (1.37 ml, 1.2equiv.) added dropwise and the solution was stirred overnight at ambient temperature. The solution was concentrated in vacuo, diluted with water, acidified to pH~1 and extracted into ethyl acetate. The extract was dried over magnesium sulphate, filtered and concentrated in vacuo at 30°C to give the acid as an oil. The recoveries of acids starting from 1g of each mixture of methyl esters were: from Peak 1, mixture 1: 0.392g; from Peak 2, mixture 1: 0.427g; from Peak 1, mixture 2: 0.433g and from Peak 2, mixture 2: 0.381g.

Chemical purity was determined by NMR and LC-MS; chiral

purity was determined by chiral HPLC as described below. ^1H NMR ($CDCl_3$) confirmed the structure of the acid and showed the presence of only a trace of the methyl ester; a low level impurity, probably the *cis* -isomer, was always present together with residual ethyl acetate.

The absolute configurations of each of the four acids produced by this technique is not known but ^{13}C NMR did show very small differences between diastereomers of the esters. Peak 1, mixture 1 and peak 2, mixture 2 were shown to be enantiomers, as were peak 2, mixture 1 and peak 1, mixture 2.

Example 3: Hydrolysis of Separated Methyl Esters

0.45g of a methyl ester (as separated in Example 2) was dissolved in 40ml of a 4:1 v/v solution of THF in water; 1.37ml of 1M lithium hydroxide solution (1.2equiv.) was added dropwise, and the solution then stirred overnight at ambient temperature. The reaction was then examined by LC-MS, which typically showed clean formation of the free acid, with only a trace of ester remaining. The reaction was concentrated down under vacuum to remove THF, and more water added; the stirred solution was treated dropwise with 1M hydrochloric acid to give pH~1, and the solution then equilibrated with ethyl acetate; the aqueous layer was removed, and the ethyl acetate layer washed with brine, dried over magnesium sulphate, filtered, and evaporated under vacuum. The residual oil was transferred to a weighed vial in a little ethyl acetate, and solvent removed under a stream of nitrogen; the sample was then placed in a drying pistol and pumped on overnight at 30°C/1mbar.

^1H NMR ($CDCl_3$) confirmed the structure of the product as the free acid, and typically showed the presence of only a trace

of methyl ester; a low level impurity, thought to be the cis-isomer, was always present, as was residual ethyl acetate.

The chiral purity of the product was assessed by re-esterifying a small sample of each of the four separated acid isomers and then analysing the esters by analytical chiral HPLC. About 5 mg of acid was dissolved in ether and treated with a freshly prepared solution of diazomethane in ether, to give a permanent yellow colour. After standing for 30 minutes at ambient temperature the solution was blown to dryness under nitrogen and re-dissolved in ethanol for chiral HPLC. The conditions used for the analysis were; analytical ChiralPak AD column (25 cm by 0.46 cm), 100% ethanol as stationary phase, flow rate of 0.25 ml/min UV detection (230 nm) at ambient temperature. Typical retention times for each isomer were: Peak 1, mixture 1: 23.5 min; Peak 2, mixture 1: 56 min; Peak 1, mixture 2: 23.7 min; Peak 2, mixture 2: 35 min. The chiral purity of each sample was essentially 100%.

Example 4: EP binding and agonism

The ability of compounds to bind to the human EP_2 receptor and their selectivity against all other EP receptors can be demonstrated in radioligand competition displacement binding experiments using cell lines stably transfected with the human EP receptors. The ability of compounds to stimulate the EP_2 receptor can be demonstrated in a second messenger cAMP functional assay, in primary human lymphocytes, monocytes or in human myometrium.

Test Details

Binding ability to human EP receptors

Membranes were prepared from cells stably transfected with human EP receptor cDNA. In brief, cells were cultured to

confluency, scraped from culture flasks, and centrifuged (800 g, 8 minutes, 4°C). Cells were twice washed in ice cold homogenisation buffer containing 10 mMTris-HCl, 1 mM EDTA.2Na, 250 mM sucrose, 1 mM PMSF, 0.3 mM indomethacin, pH 7.4, homogenised and re-centrifuged as before. The supernatant was stored on ice and pellets re-homogenised and re-spun. Supernatants were pooled and centrifuged at 40000g, 10 minutes, 4°C. Resultant membrane pellets were stored at -80°C until use.

For assay, membranes expressing human EP_4, EP_3, EP_2 or EP_1 receptors were incubated in Millipore (MHVBN45) plates containing assay buffer, radiolabelled [^{3}H]PGE_2 and 0.1 to 10 000 nM concentrations of compounds. Incubations were performed at suitable temperatures and for suitable times to allow equilibrium to be reached. Non-specific binding was determined in the presence of 10uM PGE_2. Bound and free radiolabel was separated by vacuum manifold filtration using appropriate wash buffers, and bound radiolabel was determined by scintillation counting. Constituents of each of the buffers are included in table 1 below.

The affinity or pK_i of each compound for each receptor was calculated from the concentration causing 50% radioligand displacement (IC_{50}) using the Cheng-Prusoff equation:

$$Ki = \frac{IC_{50}}{1 + \left(\frac{radioligand\ concentration}{radioligand\ KD} \right)}$$

This approach follows that set out in Kenakin, T.P., Pharmacologic analysis of drug receptor interaction. Raven Press, New York, 2nd edition.

Table 1

Receptor		EP_1	EP_2	EP_3	EP_4
Protein / well		6.5µg	8µg	5µg	5µg
Final [^{3}H-PGE_2]		3.6nM	3nM	2.5nM	1nM
Buffer	Assay	10mM MES pH6.0; 10mM $MgCl_2$; 1mM EDTA, 3uM Indomethacin	10mM MES pH6.0; 10mM $MgCl_2$; 1mM EDTA	10mM MES pH 6.0; 10mM MgCl2; 1mM EDTA, 100uM GTP-gamma-S	10mM MES pH6.0; 10mM $MgCl_2$; 1mM EDTA, 3uM Indomethacin
	Wash	10mM MES pH6.0; 10mM $MgCl_2$	10mM MES pH6.0; 10mM $MgCl_2$	10mM MES pH 6.0; 10mM $MgCl_2$	10mM MES pH6.0; 1mM EDTA

Effect of compounds on cyclase production

The following describes an in vitro assay to determine the effect of compounds on cyclase production, that is, to determine their functional efficacy at the EP_2 receptor.

Cell stimulation

HEK cells stably expressing the human EP2 receptor were used for these assays. HEK-EP2 cells were cultured in 96-well, poly-L-lysine coated plates at a density of 50,000 cells/well, and grown to confluence in humidified 95%O_2/5%CO_2 at 37°C. Culture medium was DMEM supplemented with 10% foetal bovine serum, 100U/ml penicillin, 100ng/ml streptomycin, 2.5µg/ml fungizone, 2mM glutamine, 250µg/ml geneticin and 200µg/ml zeocin.

On reaching confluence, culture media was rinsed off using DMEM with no additions, before 175µl assay buffer (DMEM containing 1mM 3-isobutyl-1-methylxanthine and 3µM indomethacin) was added to each well. This was allowed to incubate for 1hr before the cells were stimulated with the

test compounds (in triplicate) at final concentrations of 10^{-9}M to 10^{-5}M for 15 minutes. The assay was terminated by the addition of 25µl 1M hydrochloric acid. Plates were then frozen for a minimum of 12 hours or until required for radioligand displacement assay.

Radioligand displacement assay

Plates were thawed quickly at 37°C, and neutralised with 25µl 1M sodium hydroxide. 30µl of supernatant was transferred to 96-well Millipore (MAFNOB) plates coated with 0.1% Polyethylenimine. These supernatants were diluted by addition of 90µl cAMP assay buffer (50mM Tris, 5mM EDTA, pH 7.0). A cAMP standard curve (10^{-11}M to 10^{-5}M) was constructed. 15µl of 3':5'-cAMP-dependent protein kinase (final concentration 8µg/well), and 15µl [^{3}H]-cAMP (final concentration 2nM/well) were added to each well.

Plates were incubated on ice for 2 hours, before bound and free radiolabel were separated by vacuum filtration harvesting on the Millipore manifold, using ice cold water as the termination buffer. Filter plates were allowed to dry overnight, before addition of 50µl Microscint. Radioactivity was determined using the Microbeta Trilux scintillation counter. cAMP accumulation was determined from the standard curve, and the values plotted as pmoles cAMP/well.

Effect of compounds on human myometrial activity

The following describes an *in vitro* functional assay, using human myometrial smooth muscle, to determine the affinity of the test compounds at the EP_2 receptor in human tissues.

Sections of human myometrium were prepared from samples of surgically removed uterus longitudinal myometrial muscle

strips (2mm wide by 10mm long) were then cut and suspended between stainless steel hooks in organ chambers containing oxygenated (95% O_2/5% CO_2) Krebs solution at 37°C. The composition of the Krebs solution was as follows: NaCl (118.2mM), KCl (4.69mM), $MgSO_4.7H_2O$ (1.18mM), KH_2PO_4 (1.19mM), glucose (11.1mM), $NaHCO_3$ (25.0mM), $CaCl_2.6H_2O$ (2.5mM), indomethacin $3x10^{-6}$M.

Tissues were placed under a tension equivalent to 25mN and left overnight at room temperature. The following day the tissues were maintained at 37°C, washed and placed under a tension of 15mN then allowed to equilibrate for a period of at least 30 minutes. Responses were recorded using isometric transducers coupled to an Apple Macintosh computer via a MacLab interface. After 60 minutes, the muscle sections of the human myometrium were stimulated electrically (15ms pulse width, for 10s every 100s at 15V and 0.5-40Hz) using parallel platinum wire electrodes and a Multistim D330 pulse stimulator. Upon electrical stimulation, the strips of human myometrial smooth muscle responded with a rapid contraction. Once the response to electrical stimulation had stabilised (stimulated responses differed by no more than 10%), the strips were exposed to increasing concentrations of test compounds ($1x10^{-7}$ to $1x10^{-4}$M, incubated for at least 15 minutes at each concentration). At the end of the experiment, application of sodium nitroprusside (SNP, a nitric oxide donor that causes smooth muscle relaxation) ($1x10^{-4}$M) was used to produce a standard relaxatory response. To determine the affinity of the compounds, the concentration of test compound required to produce half-maximal effects (EC_{50}) was calculated, as was the maximum response (calculated as a percentage of the standard response produced with SNP).

Results

Binding ability to human EP receptors

In these tests, the affinity of the four separated stereoisomers of AH-13205 were determined, and the results are shown in figure 1 (data is shown as means±s.e for 4 experiments). The stereoisomer isolated in peak 1 of mixture 1 was shown to be the most potent, having a pKi of 7.1.

The full results of the binding tests are shown in table 2 as pKi values:

Compound	EP_2	EP_1	EP_3	EP_4
Peak 1, Mixture 1	7.1	-	5.7	5.0
Peak 2, Mixture 1	5.8	-	4.8	4.5
Peak 1, Mixture 2	6.9	-	4.8	5.1
Peak 2, Mixture 2	6.3	-	4.7	4.8
AH-13205	6.4	5.0	5.2	4.6

Table 2

From this table, it can be seen that Peak 1, mixture 2 is the most selective of the stereoisomers.

Effect of compounds on cyclase production

In these tests, the effect of the separated stereoisomers and AH-13205 on cyclase production was assessed. All the compounds showed the same maximal response, but their potency differed, as shown in Figure 2 and table 3 (data is shown as means±s.e. for 4 experiments).

Compound	Mean Log (EC_{50})	S.E.M.	Mean EC50 (nM)
Peak 1, Mixture 1	-8.01	0.22	10
Peak 2, Mixture 1	-6.49	0.19	323
Peak 1, Mixture 2	-7.25	0.19	56
Peak 2, Mixture 2	-6.39	0.24	407
AH13205	-7.49	0.29	32

Table 3

Effect of compounds on human myometrial activity

Application of AH-13205 was shown to inhibit electrically-induced contractions in human myometrium - points A, B and C correspond to the addition of increasing amounts of AH-13205 (10^{-6}, 10^{-5} and 10^{-4} M)(Figure 3). The potency of the effect was in accordance with interaction at a prostaglandin EP_2 receptor, as the vehicle containing AH-13205 was shown to have no effect (Figure 4).

The effects of the two most potent stereoisomers (Peak 1, Mixture 1 and Peak 1, Mixture 2) were investigated, and compared to the effects of AH-13205. Peak 1, Mixture 1, Peak 1, Mixture 2 and AH13205, all caused concentration-dependent inhibition of the EFS-evoked response. The pEC50s were 5.9±0.2 (n=7), 5.3±0.1 (n=6) and 5.3±0.2 (n=7) (Figure 5). There was no significant differences between the maximum inhibitory effects observed, with inhibition of EFS-induced contractions of 56±5% (Peak 1, Mixture 1), 57±2% (Peak 1, Mixture 2) and 49±5% (AH-13205). SNP caused further inhibition on top of the compounds, equivalent to 60-70% of the control EFS response. The SNP inhibitory effect was reversed over a 60-80 minutes washing period but the inhibitory effects of the compounds tested were not.

In addition, the effect of terbutaline, a β agonist, on EFS-induced contractions of myometrium was investigated, and shown to have no significant inhibitory effect on the EFS-evoked contractions (98±5% of the control EFS-induced contraction at 10^{-4}M, n=7 donors).

Example 5: Inhibition of IL-2 production

Lymphocytes are mononuclear leukocytes, which participate in specific immune responses to foreign antigens and in the manifestation of auto-immune diseases. T lymphocytes produce IL-2, a key factor for lymphocyte activation and proliferation, in response to antigen stimulation via the CD3-T cell receptor complex and the pathway involved in this response is the NF-AT. This response can be demonstrated *in vitro* by using selective monoclonal antibodies with specificity to the CD3 molecules on T cells. A lymphocyte assay was designed to model this response and to determine the effect of test compound on IL-2 production by anti-CD3-stimulated T cells isolated from peripheral blood. This assay uses a sub-optimal dose of an anti-CD3 monoclonal antibody (OKT3, 25ng/ml) immobilised to a 96-well plate to stimulate a T cell response. The level of IL-2 released into the cell culture supernatants was quantified using a standard sandwich ELISA.

Monocytes are peripheral mononuclear phagocytes that participate in inflammatory responses. TNF-alpha production by monocytes plays an important role in inflammatory responses and can cause considerable tissue damage if the level remained unchecked. Inhibition of TNF-alpha secretion by activated monocytes may provide an attractive therapy for the treatment of inflammatory conditions.

One of the most potent microbial triggers of TNF-alpha

release by monocytes is lipopolysaccharide (LPS) and this response is via the NF-KB pathway. A 96 well *in vitro* assay was established to determine the effects of test compounds on LPS-induced TNF-alpha secretion by human peripheral blood monocytes. The level of TNF-alpha in assay supernatants was quantified using a standard sandwich ELISA.

Test Details

Human peripheral blood mononuclear cells from healthy volunteers were isolated from whole blood by Ficoll-Hypaque density centrifugation and adherence to plastic. The non-adherent lymphocyte fraction was used to set up the lymphocyte assay and the adherent monocytes were then recovered by scraping and subsequently used in the monocyte assay.

Lymphocyte assay

Lymphocytes were then seeded to a 96-well plate pre-coated with anti-CD3 monoclonal antibody (OKT3) at 25 ng/ml and immediately, the test compounds (Peak 1, mixture 1; Peak 1, mixture 2; AH-13205 racemate; PGE_2) in appropriate dilutions were added to corresponding wells according to the experimental design. The plate was incubated for 24 hours at 37°C with 5% CO_2 in air and supernatants were recovered for ELISA analysis at the end of incubation period.

Monocyte assay

For the monocyte assay, the cells were plated onto 96-well plates and pre-treated for 1 hour at 37°C / 5%CO_2 with the test compound (Peak 1, mixture 1; Peak 1, mixture 2; AH-13205 racemate), followed by the addition of LPS (100ng/ml) to initiate the reaction. The plate was incubated for 24 hours and supernatants were recovered for the measurement of TNF-alpha production by ELISA.

Results

Lymphocyte assay

Figure 6 shows the results given as mean of three donors (except peak 1, mixture 2 which was tested in one donor only). These results are summarized in table 4.

Compound	Mean Log (EC_{50})	Mean EC50 (μM)
Peak 1, Mixture 1	-6.006	0.986
AH13205, racemate	-5.549	2.823
PGE_2	-7.554	0.028

Table 4

These results show that EP_2 agonists concentration-dependently inhibit IL-2 production by OKT3 activated T cells. The order of potencies in the assay is PGE_2 > Peak1, Mixture 1> AH13205 (racemate) according to their respective EC_{50} values.

Monocyte assay

Figure 7 shows the results given as mean of three donors. These results are summarized in table 5.

Compound	Mean Log (EC_{50})	Mean EC50 (μM)
Peak 1, Mixture 1	-5.749	1.783
Peak 1, Mixture 2	-5.172	6.730
AH13205, racemate	-4.704	19.780

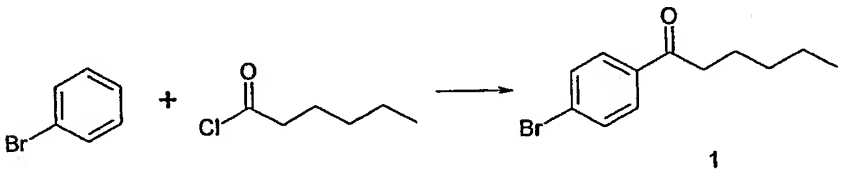
Table 5

These results show that the EP_2 agonists concentration-dependently inhibited TNF-alpha production by LPS-stimulated monocytes. The order of potencies based on their respective EC_{50} values is Peak 1, Mixture 1 > Peak 1, Mixture 2 > AH13205 (racemate).

Example 6: Stereoselective synthesis

^{1}H nmr spectra were recorded using either a Bruker AC-250 spectrometer. MS method: positive electrospray (ES^+), capillary voltage 3.25kV, cone voltage 25V.

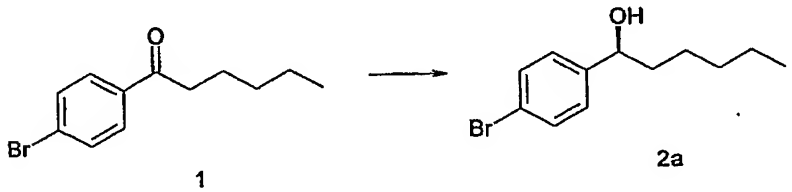
Preparation of 1-(4-bromophenyl)hexan-1-one ***(1)***



1

To a stirred ice-cooled mixture of $AlCl_3$ (83 g) and bromobenzene (200 mL) under nitrogen was added dropwise hexanoylchloride (75 mL) over a period of 30 minutes. The mixture was then heated to 80°C (external) for 1.5 hours, after which time the solution had turned a deep red. The mixture was then allowed to cool before being poured into 600mL ice water and then extracted with DCM (800 mL). The organic extracts were then washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. The concentrate was then treated with *iso*-hexane (1 L) and left in the freezer overnight, wherein crystallization took place. The slightly off-white solid was filtered and washed with more cold hexane, to yield the title compound (84g). Shown to be adequately pure by nmr and tlc.

Preparation of (S)-(-)-1-(4-bromphenyl)hexan-1-ol ***(2a)***



1 2a

BH_3.THF (1 M in THF, 234 mL, 234 mmol) was stirred under nitrogen and cooled to -10°C before being treated with (R)-2-methyl-CBS-oxazaborolidine (24 mL, 24 mmol). After being

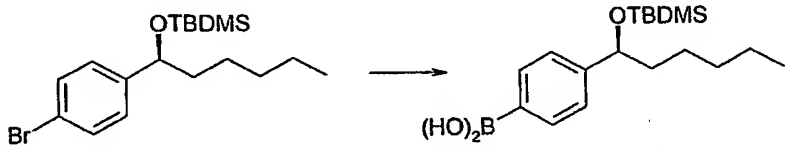
left stirring for 20 minutes, 1-(4-bromophenyl)hexan-1-one (1) (48.3 g) was added as a solution in THF (379 mL) over a period of 1 hour, and thereafter left for a further 20 minutes before quenching carefully with MeOH (100 mL) - H_2 evolves. Advisable to perform the quench at RT since MeOH reacts slowly at -10°C. The mixture was then concentrated *in vacuo*, then redissolved in MeOH (300 mL) and treated with HCl (2 M in Et_2O, 40 mL). The solution was stirred for 5 minutes before concentrating *in vacuo*, triturating with Et_2O and removing the solid by filtration. The mother liquors were again concentrated *in vacuo* then recrystallized from hexane (480 mL, 10 vol.) at -10°C to yield the title compound as a fluffy white solid (20.7 g).

Preparation of (S)-(-)-1-O-TBDMS-1-(4-bromphenyl)hexane ***(3a)***

2a 3a

To a stirred solution of (S)-(-)-1-(4-bromphenyl)hexan-1-ol (**2a**) (2.57, 10 mmol) in DMF (40 mL) at 40°C under nitrogen was added imidazole (2.74g) and TBDMS-Cl (3.03 g, 20 mmol). The mixture was left for 1 hour before quenching with H_2O (5 mL) and leaving for 5 minutes. The mixture was then partitioned between 1 M HCl (aq) (200 mL) and *iso*-hexane (200 mL). The aqueous extract was washed with a further 50 mL of hexane before combining all organic extracts, washing with brine, drying ($MgSO_4$) and concentrating *in vacuo* until all TBDMS-OH disappears (high-vac). This yielded the title compound, which was consistent by nmr, as a clear liquid/oil (3.5 g).

Synthesis of (S)-(-)-1-O-TBDMS-1-(phenyl-4-boronate)hexane (8)



3a 8

To a stirring solution of 1-*O*-TBDMS-1-(4-bromphenyl)hexane (**3a**) (3.17 g, 8.58 mmol) in THF (32 mL) under nitrogen at -78°C was slowly added BuLi (2.5 M in hexanes, 3.43 mL, 8.58 mmol). After leaving to stir for 10 minutes the mixture was treated with tri*iso*propylborate at -78°C. The mixture was then warmed to room temperature and treated with 5M KOH (aq) (12.86 mL) and left stirring for 30 minutes. The solution was then acidified to pH ~6 with AcOH, before concentrating *in vacuo*, then partitioning between EtOAc and water. The organics were dried ($MgSO_4$), concentrated *in vacuo* then purified by silica column chromatography [Hexane to EtOAc : Hexane (3 : 7) as eluent] to yield a clear oil which solidified upon standing to a white solid (1.5 g). δ_H ($CDCl_3$, 250 MHz) -0.10 (3 H, s, Si-Me), 0.06 (3 H, s, Si-Me), 0.80-1.00 (3 H, m, CH_3), 0.92 (9 H, s, Si-^{t}Bu), 1.3-1.8 (8 H, m, 4 x CH_2), 4.71 (1 H, brt, CHOTBDMS), 7.44 (2H, d, *J* 7.9, 2 x 2-Ar(H)), 8.19 (2H, d, *J* 7.9, 2 x 3-Ar(H)).

Key to Figures

Figure 1

■	Peak 1, mixture 1
▲	Peak 2, mixture 1
□	Peak 1, mixture 2
o	Peak 2, mixture 2
●	AH-13205 (racemate)

Figure 2

■	Peak 1, mixture 1
▲	Peak 2, mixture 1
●	Peak 1, mixture 2
o	Peak 2, mixture 2
□	AH-13205 (racemate)

Figure 4

□	Vehicle alone
■	Vehicle + AH13205

Figure 5

□	Peak 1, mixture 1
■	Peak 1, mixture 2
o	AH-13205 (racemate)

Figure 6

■	Peak 1, mixture 1
□	Peak 1, mixture 2
o	AH-13205 (racemate)
▲	PGE_2

Figure 7

■	Peak 1, mixture 1
□	Peak 1, mixture 2
●	AH-13205 (racemate)

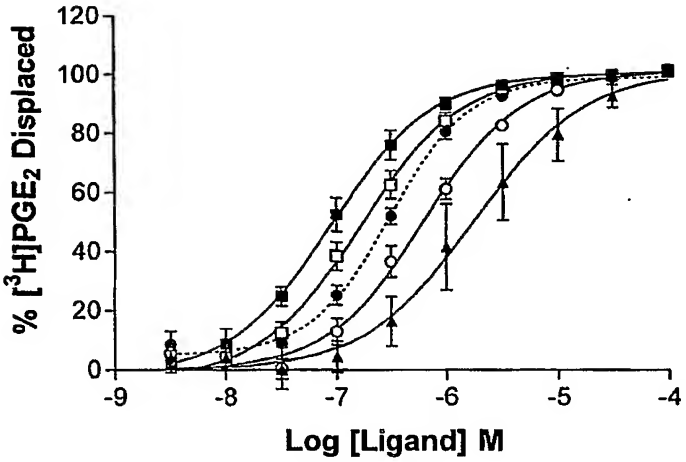


Fig. 1

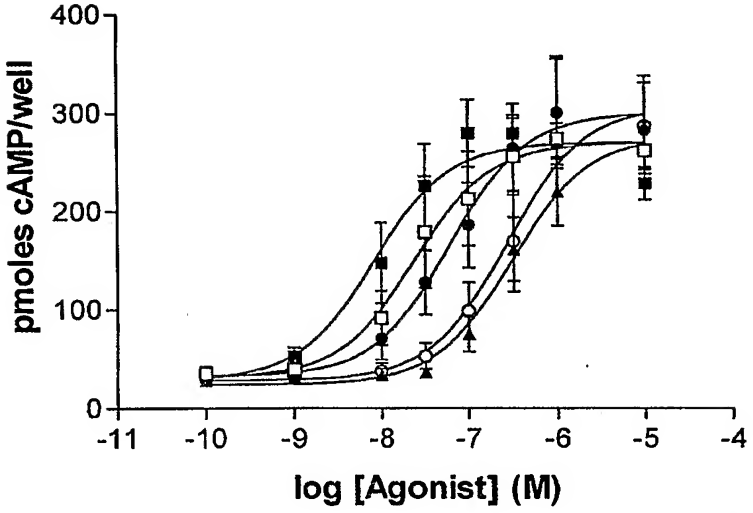


Fig. 2

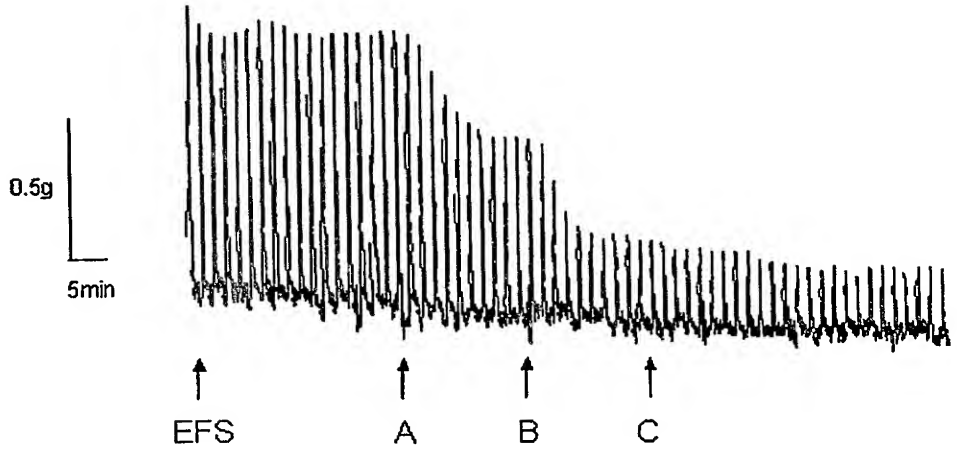


Fig. 3

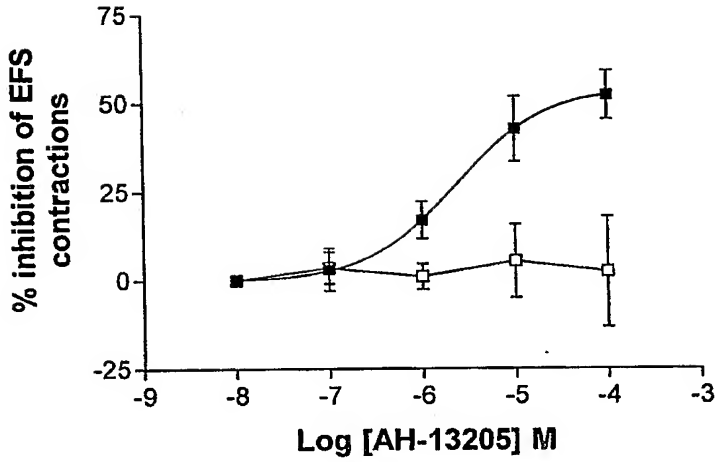


Fig. 4

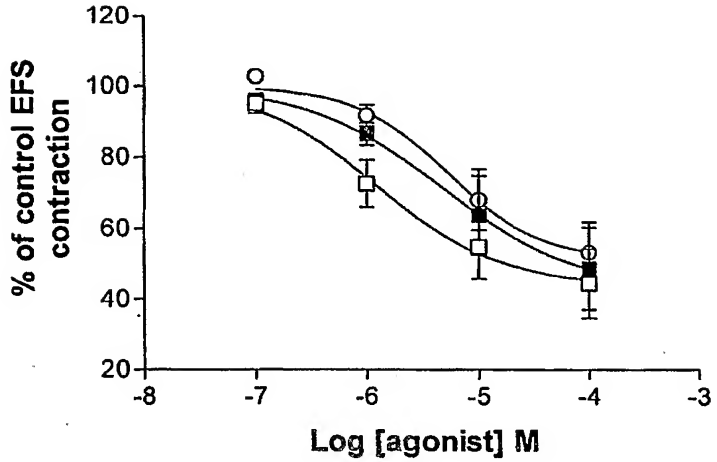


Fig. 5

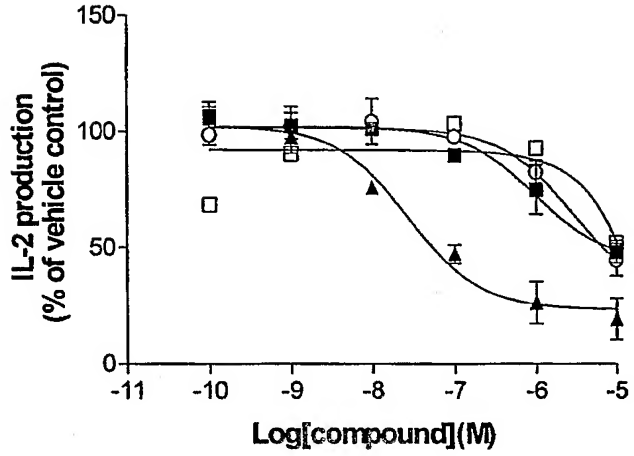


Fig. 6

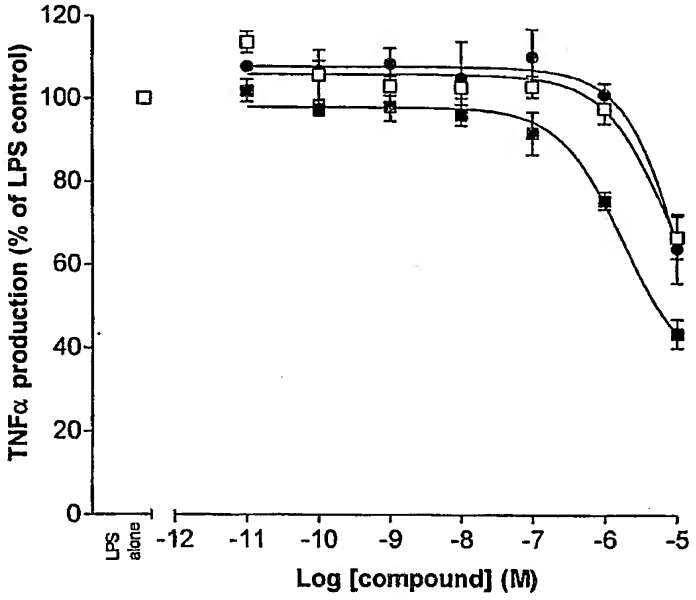


Fig. 7